Data Evaluation Report on the Toxicity of Methomyl Technical to Fish, Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 45013202

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D264036
OECD Data Point	
EPA MRID	45013202
EPA Guideline	72-4(a)

Test material: **Purity:** 98.6%

Common name: Methomyl technical

Chemical name: IUPAC S-methyl N-(methylcarbamoyloxy)thioacetimidate
CAS name: Not reported
CAS No.: 16752-77-5
Synonyms: DPX-X1179, DPX-X1179-512 (Batch Number)
EPA PC Code: 090301
Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]

Primary Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 7/26/01

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Signature:
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Biologist, OPP/EFED/ERBIV
Date: 5/7/04

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 090301

CITATION: Boeri, R.L., J.P. Magazu and T.J. Ward. 1998. Methomyl Technical: Flow-Through Early Life Stage Toxicity to the Sheepshead Minnow, *Cyprinodon variegatus*. Unpublished study performed by T.R. Wilbury Laboratories, Inc. Marblehead, Massachusetts. Report Number DuPont - 1156. Study submitted by E.I. du Pont de Nemours and Company, Wilmington, Delaware. Study initiated August 14, 1998 and completed November 25, 1998.

EXECUTIVE SUMMARY:

The 36-day chronic toxicity of Methomyl technical to the early life stage of sheepshead minnow (*Cyprinodon variegatus)* was studied under flow-through conditions. Fertilized eggs/embryos (80 embryos/treatment), <24 hours old, were exposed to Methomyl technical. Nominal concentrations included a negative control, 0.066, 0.13, 0.25, 0.50 and 1.0 mg a.i./L. Mean measured concentrations were 0.066, 0.13, 0.26, 0.49 and 1.0 mg a.i./L. The test system was maintained at 29.0 to 31.0°C and a pH of 7.7 to 7.9. Observed endpoints included the time to hatch start and end, survival of embryos and juveniles, the number and percent of healthy embryos after 48 hours of exposure, the number and percent of healthy embryos hatched, length and wet weight of surviving fish, time to first feeding, and sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in appearance or behavior). The most sensitive measured biological endpoints were the mean wet weight and total length of surviving fish. Exposure of embryonic, larval, and juvenile sheepshead minnows to Methomyl technical for 36 days resulted in a NOEC of 0.26 mg a.i./L, a LOEC of 0.49 mg a.i./L, and a MATC of 0.36 mg a.i./L Methomyl for both total length and wet weight. Fish with deformed bodies and fish exhibiting lethargy and erratic swimming were noted at 1.0 mg/L on days 4 through 16, but were not observed at any other time during the test. No other sublethal effects (other than size differences) were noted at any other time or concentration.

This toxicity study is scientifically sound and is consistent with the guideline requirements for an early life stage toxicity study using Sheepshead minnow (§72-4a). **This study is classified as CORE**.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): <24 hours old at test initiation
Test Type (Flow-through, Static, Static Renewal): Flow-through

Endpoint(s) Affected: Survival by 36 days, fish total length and wet weight

Survival by 36 days:
NOEC: 0.49 mg a.i./L
LOEC: 1.0 mg a.i./L

Total length:
NOEC: 0.26 mg a.i./L
LOEC: 0.49 mg a.i./L

Wet weight:
NOEC: 0.26 mg a.i./L
LOEC: 0.49 mg a.i./L

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures of the U.S. Environmental Protection Agency (1985, 1988) and ASTM (1992).

COMPLIANCE: Signed and dated GLP and no data confidentiality claims statements were provided.

Deviations:

1) The dilution water hardness was not reported.

2) The pH level during the study was slightly higher than recommended (7.7-7.9 measured; 7.2-7.6 recommended).

3) Temperature was higher than recommended for sheepshead minnow (30 ± 1°C measured; 25 ± 2°C recommended).

4) There were only two replicates per treatment level, instead of the four replicates per treatment level recommended by US EPA.

5) Total residual chlorine was measured in the dilution water on day 21 of the definitive test, rather than at the start of the test, due to an oversight.

6) Fish were not fed the last 24 hours of the test.

7) The endpoint, time till swim-up, was not measured.

A. MATERIALS:

1. Test Material Methomyl technical

Description: Off-white powder

Lot No./Batch No. : DPX-X1179-512

Purity: 98.6%

Stability of Compound: No insoluble material was observed in any test vessel. Mean measured concentrations of Methomyl were 98-104% of nominal concentrations and stable throughout the test. OECD requirements not reported.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored in the dark at room temperature.

2. Test organism:

Species: Sheepshead minnow (*Cyprinodon variegatus)*

EPA requires any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See SEP for complete listing.

Age /embryonic stage at test initiation: <24 hours old at test initiation
EPA requires fish embryos 2 to 24 hours old.
Method of collection of the fertilized eggs: Eggs were collected during natural spawning of up to 33 pairs of conditioned adult fish
Source: Aquatic BioSystems, Inc., Fort Collins, Colorado

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding study: A range-finding study was conducted under static-renewal conditions during Sept. 3 to 14, 1998 with sheepshead minnow embryos which were less than 24 hours old. Nominal concentrations were 0 mg/L (control), 0.010, 0.050, 0.10, 0.50, 1.0 and 10 mg/L. After 11 days of exposure (10 organisms were exposed to each concentration) there was 20%, 60%, 90%, 80%, 100%, 100%, and 0% survival in the control, 0.010, 0.050, 0.10, 0.50, 1.0 and 10 mg/L exposure levels, respectively.

b) A definitive test (Definitive Study #1) was attempted under flow-through conditions during September 18 to 22, 1998 with sheepshead minnow embryos which were less than 24 hours old. Nominal concentrations were 0 mg/L, 0.54, 1.0, 2.0, 4.0, and 8.0 mg/L. After 4 days of exposure, there was 98, 96, 88, 89, 59, and 25% survival in the control, 0.54, 1.0, 2.0, 4.0, and 8.0 mg/L, respectively. Measured concentrations collected on day 0 from each test vessel ranged from 95 to 101% of nominal. Because affected fish (exhibiting lethargy, erratic swimming, deformed bodies, and/or smaller than controls) were observed at all tested concentrations, the test was terminated and repeated.

c) Definitive Study #2 conducted from September 25 to October 31, 1998.

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions: (same as test or not) Feeding (type, source, amount given, frequency): Health: (any mortality observed)	NA, spawned and fertilized off-site	Eggs were collected by Aquatic Biosystems, Inc. during natural spawning of up to 33 pairs of conditioned adult fish, which were introduced into spawning tanks less than 24 hours before test initiation. Fertilized eggs were shipped to T.R. Wilbury Laboratories by overnight express delivery. Fertilization was confirmed at test initiation by direct visual observation with a microscope and the embryos were free of apparent disease, injuries, and abnormalities at the beginning of the test.
Number of fertilized eggs/embryos in each treatment at test initiation	Two cages, each containing 20 embryos, were suspended in each of 2 replicates (80 embryos total)	*EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure*

Parameter	Details	Remarks *Criteria*
Concentration of test material: nominal: measured:	 0.0, 0.066, 0.13, 0.25, 0.50 and 1.0 mg a.i./L 0.0, 0.066, 0.13, 0.26, 0.49 and 1.0 mg a.i./L	Measured concentrations are the average of concentrations measured over the 36 day test. *EPA requires a minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.* *- Toxicant conc. must be measured in one tank at each toxicant level every week.* *- One concentration must adversely affect a life stage and one concentration must not affect any life stage.* *OECD requires 5 concentrations spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution must be within ± 20% of the mean measured values.*
Solvent (type, percentage, if used)	NA	*EPA requires that solvent should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD requires that solvent must have no effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 ml/L.*
Number of replicates control: solvent control: treated ones:	 2 NA 2	There were only two replicates per concentration. *EPA requires 4 replicates per concentration* *EPA/OECD require solvent control when a solubilizing agent has been used.*

Parameter	Details	Remarks *Criteria*
Test condition: static renewal/flow through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 flow-through intermittent flow proportional diluter 10 volume additions per day NA	The volume of media delivered to a replicate test vessel was within 10% of the other replicate. *Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be 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%..*
Aeration, if any	Dilution water was aerated prior to use, but test solutions were not aerated.	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	36 days	*EPA requires 32 days*
Embryo cups, if used type/material: (glass/stainless steel) size: fill volume:	 glass cylinders (8 cm high x 8 cm diameter) Not provided	Glass cylinders were closed on one end with 350 µm Nitex® screen *EPA requires 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
Test vessel type/material: (glass/stainless steel) size: fill volume:	 glass aquaria 9 L (15 x 30 x 20 cm) 8 L of test solution; water depth approx. 17 cm.	 *EPA/OECD requires all glass or glass with stainless steel frame.*

Parameter	Details	Remarks *Criteria*
Source of dilution water	Natural sea water collected from the Atlantic Ocean at T.R. Wilbury laboratories in Marblehead, Massachusetts and adjusted to a salinity of 15-16 ppt with deionized water.	A representative sample of undiluted natural seawater used to formulate dilution water for toxicity tests was analyzed for the presence of pesticides, PCBs and toxic metals by Lancaster Laboratories, Inc., as part of routine water quality testing conducted biannually. *EPA requires natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*
<u>Water parameters:</u> hardness: pH: dissolved oxygen: temperature (s) (record all the temperatures used for different life stages): photoperiod: salinity (for marine or estuarine species): other measurements: interval of water quality measurements:	 Not reported 7.7-7.9 6.3-8.0 mg/L 29.0-31.0°C NA 16:8 15-16 daily; temperature recorded continuously in one control test vessel during the study	Hardness is not reported. pH is slightly higher than recommended (7.2-7.6). Temperature is higher than recommended for sheepshead minnow (25 ± 2°C). *EPA requires hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended. DO must be measured at each conc. at least once a week; freshwater parameters in a control and one concentration must be analyzed once a week.* *Temperature depends upon test species; should not deviate by more than 2°C from appropriate temperature.* *OECD requires DO concentration between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*

Parameter	Details	Remarks *Criteria*
Post-hatch details: when the post-hatch period began: number of hatched eggs / treatment released to the test chamber: on what day the hatched fish were released from the incubation cups to the test chamber	 Day 4 of exposure 15 per replicate At day 4 of the exposure, hatched fish in all test vessels were thinned to 15 per replicate	>90% hatch in each control replicate *EPA requires % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.*
Post-hatch Feeding: start date: type/source of feed: amount given: frequency of feeding:	 Day 5 Fed newly hatched *Artemia salina* nauplii *ad libitum* 2-3 x daily except during the final 24 hours	
Stability of chemical in the test system	No insoluble material was observed in any vessel during the test. Measured test concentrations were 98 to 104% of nominal concentrations and were stable over the test duration.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	98 to 104% of nominal samples collected on days 0, 7, 14, 21, 28, 35 and 36 0.0050 mg/L 0.010 mg/L	
Positive control {if used, indicate the chemical and concentrations}	NA	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	 80 per level Fertilization was confirmed upon arrival at the laboratory at test initiation by direct visual analysis via a microscope	
Other parameters, if any	NA	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Time to hatch start and end, survival of embryos and juveniles, the number and percent of healthy embryos after 48 hours of exposure, the number and percent of healthy embryos hatched, length and wet weight of surviving fish, time to first feeding and sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in appearance or behavior).	The time to hatch start and end were identical for all tested concentrations, and statistical analysis was not warranted. *EPA minimally requires:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and juveniles;* *- Time to swim-up (if approp.);* *- Measurement of growth;* *- Incidence of pathological or histological effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry: swim-up behavior: growth measurements: embryonic development: other sublethal effects	 Daily Daily Daily Not reported At test termination At test initiation Daily	
Water quality was acceptable (Yes/No)	1) Hardness was not reported 2) pH was slightly higher than recommended (7.7-7.9 measured; 7.2-7.6 recommended) 3) Temperature was higher than recommended (30 ± 1 °C measured; 25 ± 2 °C recommended).	
Were raw data included?	Yes	
Other observations, if any	NA	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Average percent survival at 32 days post hatch decreased with increasing concentrations. Average percent survival was 100, 97, 90, 93, and 34% in the 0.066, 0.13, 0.26, 0.49, and 1.0 mg a.i./L treatment groups, respectively,

compared to 97% in the control group. The LC_{50} was 0.838 mg a.i./L, the NOEC was 0.49 mg a.i./L, and the LOEC was 1.0 mg a.i./L.

Table 3: Effect of Methomyl technical on egg hatching and survival at different life stage of fish.

[1]Treatment (mg a.i./L) [record measured and nominal concentrations used	Egg hatched/embryo viability			Time to hatch[3]			Juvenile-survival on day 36[4]	
	No. of eggs at study initiation	hatch/embryo viability		day 2 (begin%)	day 3 (%)	day 4 (end%)	No. dead	% mortality
		No.*	%					
Control (dilution water only), if used	80	73	91.3	0	71.3	91.3	1	3.3
Solvent control, if used	NA	NA	NA	NA	NA	NA	NA	NA
0.066 (0.066)	80	74	92.5	0	48.8	92.5	0	0.0
0.13 (0.13)	80	73	91.3	0	61.3	91.3	1	3.3
0.26 (0.25)	80	73	91.3	0	53.8	91.3	3	10
0.49 (0.50)	80	78	97.5	0	91.3	97.5	2	6.7
1.0 (1.0)	80	76[2]	95.0	0	31.3	95.0	20	66.7
NOEC mg a.i./L			1.0	1.0		1.0		0.49
EC_{50} mg a.i./L			0.68	>1.0		0.68		0.84
Positive control, if used mortality: EC_{50}:	NA	NA	NA	NA	NA	NA	NA	NA

[1] Nominal concentrations are in parentheses.
[2] All surviving fish were deformed and exhibited erratic swimming.
[3] The reviewer determined the % of embryos hatched at days 2, 3 and 4 in order to assess treatment effects on time to hatch.
[4] After thinning on day 4, 30 fish per treatment level were observed.
* Number of live fish by day 4, last day of hatch. Fish were thinned after counting.

Table 4: Effect of Methomyl technical on growth of juvenile fish

[1]Treatment (mg a.i./L) [record measured and nominal concentrations used]	Swim-up			Growth -length (cm)	Growth-wet weight (g)
	day x1	day x2	day xn		
Control (dilution water only), if used	NR[2]	NR	NR	22.4	203
Solvent control, if used	NA	NA	NA		
0.066 (0.066)	NR	NR	NR	21.2	184
0.13 (0.13)	NR	NR	NR	21.2	195
0.26 (0.25)	NR	NR	NR	21.2	192
0.49 (0.50)	NR	NR	NR	19.5	172
1.0 (1.0)	NR	NR	NR	15.1	92
NOEC	NR	NR	NR	0.26	0.26
EC_{50}	NR	NR	NR	NR	NR
Positive control, if used mortality: EC_{50}:	NA	NA	NA	NA	NA

[1] Nominal concentrations are in parentheses.
[2] NR = No data Reported for this endpoint.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Sublethal effects observed as fish with deformed bodies and fish exhibiting lethargy and erratic swimming were noted at 1.0 mg a.i./L on days 4 through 16 of the definitive test. No other sublethal effects (other than size differences) were noted at any other time or concentration.

The most sensitive measured biological endpoints were the mean wet weight and total length of surviving fish at the end of the test. The NOEC for fish exposed for 36 days to Methomyl Technical was 0.26 mg a.i./L, the LOEC was 0.49 mg/L, and the MATC was 0.36 mg a.i./L Methomyl.

Table 5: Sub-lethal effect of Methomyl Technical on the Sheepshead minnow.

[1]Treatment (mg a.i./L) [indicate if measured or nominal concentrations used]	% deformed larvae (at 48 hours)	Behavioral effects (% normal at hatch)	Behavioral effects (% live normal at day 14)	Toxicity symptoms (% live normal at day 32)	Toxicity symptoms (specify)
Control (dilution water only), if used	2	92	100	97	

[1]Treatment (mg a.i./L) [indicate if measured or nominal concentrations used]	% deformed larvae (at 48 hours)	Behavioral effects (% normal at hatch)	Behavioral effects (% live normal at day 14)	Toxicity symptoms (% live normal at day 32)	Toxicity symptoms (specify)
Solvent control, if used	NA	NA	NA	NA	
0.066 (0.066)	3	93	100	100	
0.13 (0.13)	2	92	97	97	
0.26 (0.25)	2	92	93	90	
0.49 (0.50)	1	98	97	93	
1.0 (1.0)	2	0	44	34	
NOEC mg a.i./L	1.0	0.49	0.49	0.49	
LOEC mg a.i./L	> 1.0	1.0	1.0	1.0	
Positive control, if used % sublethal effect: NOEC:	NA	NA	NA	NA	NA

[1] Nominal concentrations are in parentheses.

C. REPORTED STATISTICS:

Statistical Method: Results of the toxicity test were interpreted by standard statistical techniques (U.S. EPA, 1988), when warranted. Computer methods (TOXSTAT 3.5; Gulley, et al., 1996) were used to analyze the data. Data that were statistically analyzed included: 1) percent of healthy and unhealthy larvae and juveniles hatched, survival at 48 hours, and at 7, 14, 21, 28, and 32 days after hatch, 2) sublethal effects at hatch and 7 days after hatch, 3) the mean total length of surviving fish at the end of the test, and 4) the mean wet weight of surviving fish at the end of the test. The time to first feeding was not statistically analyzed because all fish fed when first presented with food. The time to hatch start and end were identical for all tested concentrations, and statistical analysis was not warranted. Because no sublethal effects other than visual size differences (statistically analyzed as weight and length) were observed from day 13 post hatch through the end of the test, sublethal effects for days 14, 21, 28, and 32 days after hatch were statistically analyzed using survival data.

The Shapiro-Wilke's or Chi-square tests were used to determine that data were normally distributed, and Cochran's test was used to determine that variances were homogeneous. A one-way analysis of variance (ANOVA) and Dunnett's test was then used to compare treatment and control means. All calculations were performed using mean measured concentrations of the active ingredient. Survival and sublethal effects data were arc sine [square root (Y)] transformed prior to statistical analysis.

The EC50 values and 95% confidence limits were calculated for days 4 (hatch), 7, 14, 21, 28 and 32 days post hatch by the binomial or probit method (Stephan, 1983). The 48 hour EC50 value could not be calculated because

there was greater than 50% survival of unaffected embryos at all tested concentrations. The slope of the concentration response curve could not be calculated with this data set.

The no observed effect concentration (NOEC) is the highest tested concentration at which a measured biological parameter is not statistically different (at the 95% confidence interval) than the control. The lowest observed effect concentration (LOEC) is the lowest tested concentration at which any measured biological parameter is statistically different from the control and above which all concentrations are significantly different. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and the LOEC.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Percent survival at 48 hours and at hatch as well as percent live normal at 48 hours and time to hatch were assessed visually for significant biological effects because initial inspection of the data indicated no reduction in any of these measured endpoints compared to their respective controls. Survival by day 36 data were arc sin transformed and evaluated using ANOVA-Dunnet's to determine treatment differences from control using. Total length and wet weight were also evaluated using ANOVA-Dunnet's to determine treatment differences from control. See Appendix 1 for output of reviewer's statistical verification.

Survival by 36 days:

NOEC: 0.49 mg a.i./L

LOEC: 1.0 mg a.i./L

Total length:

NOEC: 0.26 mg a.i./L

LOEC: 0.49 mg a.i./L

Wet weight:

NOEC: 0.26 mg a.i./L

LOEC: 0.49 mg a.i./L

E. STUDY DEFICIENCIES:

Only two replicates per treatment level were used, instead of the four replicates per treatment level recommended by US EPA. The other deviations/deficiencies previously listed were considered to be minor.

F. REVIEWER'S COMMENTS:

Although deficiencies/deviations occurred in this study, the study is scientifically sound. As a result, the reviewer concludes that the deficiencies did not adversely affect the results of the study and do not impact the acceptability of the study. The reviewer's conclusions are in agreement with those of the study author.

G. CONCLUSIONS:

This toxicity study is scientifically sound and is consistent with the guideline requirements for an early life stage toxicity study using Sheepshead minnow (§72-4a). **This study is classified as CORE.**

Endpoint(s) Affected: Survival by 36 days, fish total length and wet weight

Survival by 36 days:
NOEC: 0.49 mg a.i./L
LOEC: 1.0 mg a.i./L

Total length:
NOEC: 0.26 mg a.i./L
LOEC: 0.49 mg a.i./L

Wet weight:
NOEC: 0.26 mg a.i./L
LOEC: 0.49 mg a.i./L

III. REFERENCES:

ASTM. 1992. Standard Practice for Conducting Early Life-Stage Toxicity Tests with Fishes.

Bigelow, H.B., and W.C. Schroeder. 1953. Fishes of the Gulf of Maine. Fisheries Bulletin 74. Fishery Bulletin of the Fish and Wildlife Service, Volume 53. U.S. Government Printing Office, Washington, D.C.

Gulley, D.D., A.M. Boelter, and H.L. Bergman. 1996. TOXSTAT Version 3.5 Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie.

Kuntz, A. 1916. Notes on the Embryology and Larval Development of Five Species of Teleostean Fishes. Bill. U.S. Bur. Fish. 34(831): 409-429.

Japan MAFF. 1984. Good Laboratory Practice Standards. 59 NohSan No. 3850.

OECD. 1992. The OECD Principles of Good Laboratory Practice. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. Environmental Monograph No. 45. OECD/GD(92)32. Paris.

Stephan, C.E. 1983. Computer Methods for the Calculation of LC50 Values. Personal Communication.

U.S. EPA. 1985. FIFRA. Hazard Evaluation Division Standard Evaluation Procedure-Fish Early Life Stage. Hazard Evaluation Division, Office of Pesticide Programs, Washington, DC.

U.S. EPA. 1988. FIFRA. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, 72-4. Fish Early Life Stage and Aquatic Invertebrate Life-Cycle Studies. Ecological Effectes Branch, Hazard Evalution Division, Office of Pesticide Programs, Washington, D.C. March, 1988.

U.S. EPA. 1993a. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rules.

U.S. EPA. 1993b. 40 DRF Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards. Final Rule.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION :

Survival by 36 days:

Title: 13202sur
File: 13202SUR. Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.9368	0.1874	33.2529
Within (Error)	6	0.0338	0.0056	
Total	11	0.9706		

(p-value = 0.0003)

Critical F = 8.7459 (alpha = 0.01, df = 5,6)
= 4.3874 (alpha = 0.05, df = 5,6)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: 13202sur
File: 13202SUR. Transform: ARC SINE(SQUARE ROOT(Y))

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	TRANS T STAT	SIG 0.05
1	control	1.3722	0.9650		
2	0.066	1.4413	1.0000	-0.9213	
3	0.13	1.3722	0.9650	0.0000	
4	0.26	1.2525	0.9000	1.5947	
5	0.49	1.3030	0.9300	0.9213	
6	1.0	0.6156	0.3350	10.0801	*

Dunnett critical value = 2.8300 (1 Tailed, alpha = 0.05, df = 5,6)

Title: 13202sur
File: 13202SUR. Transform: ARC SINE(SQUARE ROOT(Y))

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.066	2	0.1207	12.6	-0.0350
3	0.13	2	0.1207	12.6	0.0000
4	0.26	2	0.1207	12.6	0.0650
5	0.49	2	0.1207	12.6	0.0350
6	1.0	2	0.1207	12.6	0.6300

Total length:

Title: 132021n
File: 132021LN. Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	69.1600	13.8320	24.9976
Within (Error)	6	3.3200	0.5533	
Total	11	72.4800		

(p-value = 0.0006)

Critical F = 8.7459 (alpha = 0.01, df = 5,6)
= 4.3874 (alpha = 0.05, df = 5,6)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: 132021n
File: 132021LN. Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	control	22.4500	22.4500		
2	0.066	21.1500	21.1500	1.7476	
3	0.13	21.2000	21.2000	1.6804	
4	0.26	21.2500	21.2500	1.6132	
5	0.49	19.4500	19.4500	4.0330	*
6	1.0	15.1000	15.1000	9.8808	*

Dunnett critical value = 2.8300 (1 Tailed, alpha = 0.05, df = 5,6)

Title: 132021n
File: 132021LN. Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.066	2	2.1051	9.4	1.3000
3	0.13	2	2.1051	9.4	1.2500
4	0.26	2	2.1051	9.4	1.2000
5	0.49	2	2.1051	9.4	3.0000
6	1.0	2	2.1051	9.4	7.3500

Wet weight:

Title: 13202wt
File: 13202WT . Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	16975.6667	3395.1333	30.8648
Within (Error)	6	660.0000	110.0000	
Total	11	17635.6667		

(p-value = 0.0003)

Critical F = 8.7459 (alpha = 0.01, df = 5,6)
= 4.3874 (alpha = 0.05, df = 5,6)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: 13202wt
File: 13202WT . Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	control	202.5000	202.5000		
2	0.066	183.5000	183.5000	1.8116	
3	0.13	195.0000	195.0000	0.7151	
4	0.26	192.5000	192.5000	0.9535	
5	0.49	172.0000	172.0000	2.9081	*
6	1.0	91.5000	91.5000	10.5834	*

Dunnett critical value = 2.8300 (1 Tailed, alpha = 0.05, df = 5,6)

Title: 13202wt
File: 13202WT . Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.066	2	29.6813	14.7	19.0000
3	0.13	2	29.6813	14.7	7.5000
4	0.26	2	29.6813	14.7	10.0000
5	0.49	2	29.6813	14.7	30.5000
6	1.0	2	29.6813	14.7	111.0000