UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

ENVIRONMENTAL FATE AND EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

Chemical: Methidathion
PC Codes: 100301
Barcode: D287592
Date: September 24, 2008

Subject: Guideline studies on the effect of Methidathion on the early life stage of fathead minnows.

From: James Goodyear
Ecological Effects Biologist
Environmental Review Branch 3
Environmental Fate and Effects Division 7507P

To: Tom Meyers, RB2
Risk Manager Reviewer
Special Review and Reregistration Division 7508P

Through: Mark Corbin, Branch Chief
Environmental Review Branch 3
Environmental Fate and Effects Division 7507P

The registrants of Supracide® (Methidathion) have submitted a study on its 35-day chronic toxicity of to the early life stage of Fathead Minnows (*Pimephales promelas*) under flow-through conditions. The study was originally submitted in 1984, when it was classified as "Invalid," because no data or statistical analysis was included. The new submission included these parts. It was reclassified ACCEPTABLE.

Fertilized eggs (140 eggs/level, 4 reps/level, 35 eggs/rep; <48 hrs old) of fathead minnow were exposed time-weighted concentrations were <0.24-<0.42 (<LOQ; negative and solvent controls), 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L. The test system was maintained at 24 to 27 °C and a pH of 7.9-8.3. The 35-day EC_{50} and NOAEC values, based on survival, total length and wet weight, were >12 and 6.3 µg ai/L, respectively. The sub-lethal effects included reduced juvenile survival and inhibitions of total length and dry weight. The most sensitive end points were percent survival, total length and wet weight.

The hardness and alkalinity were five times the level that EPA allowed when the study was originally submitted, but these levels are now allowed, because they meet the OECD protocol.

This toxicity study is scientifically sound and satisfies the requirements of §72-4a for an early life toxicity study with *Pimephales promelas*. It is classified as **Acceptable.**

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Results Synopsis

Test Organism Size/Age(mean Weight or Length): Eggs, <48 Hrs
Test Type (Flow-through, Static, Static Renewal): Flow-Through

Percent Survival (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Total Length (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Wet Weight (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Endpoint(s) Affected: Percent Survival, Total Length and Wet Weight

Toxicity of Methidathion to Fish
Early Life Stage

Data Requirement: PMRA Data Code {...} OECD Data Point {....}
EPA Barcode D287592
EPA MRID 001573-53 & 458227-01
EPA Guideline 72-4a
For control of certain insects of artichokes; certain citrus, fruits and nuts; olives; safflowers; sunflowers; cotton; nursery stock;

Test Material: Supracide® 2E **Purity (%):** 97.2%
Common name: Methidathion Reg. No. 10163-236
Chemical name: IUPAC: Methidathion: 0, O—dimethyl phosphorodithioate, Z—ester with 4- 4-(mercaptotnethyl)-2-methoxy-z2-1,3,4-thiadiazo1in-5-one CAS name CAS No. 950-37-8 Synonyms-
"Supracide 2E contains 2 lbs. ai per gallon;" "1 pt. Supracide 2E = 1/4 lb ai."

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental Inc. **Date:** 11/26/06

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental Inc. **Date:** 12/01/06

Primary Reviewer: James Goodyear, Ph.D. **Signature:**

Biologist, EPA/OPP/EFED/ERB3 **Date:** 2008 Sep 23

Reference/Submission No.: {.....} **Company Code** [For PMRA] **Active Code** [For PMRA]
Use Site Category [For PMRA] **EPA PC Code** 100301
Date Evaluation Completed: 2008 Sep 23

CITATION:: McAllister, W.; L. Franklin, V. Knox, 1984. Early Life Stage Toxicity of Supracide® to fathead minnows (*Pimephales promelas*) in a flow-through system. Final Report #31330. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 30 pp. MRID 001573-53

MRID 458227-01: Winkler, V. (2002) Methidathion: Raw data for fathead minnow Early Life Stage study: Lab Project Number: VW 070902: 031330: 7809. Unpublished study prepared by Gowan Company. 337 pp.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 35-day chronic toxicity of Supracide® (Methidathion) to the early life stage of Fathead Minnows (*Pimephales promelas*) was studied under flow-through conditions. The study was originally submitted in 1984, when it was classified as "Invalid," because no data or statistical analysis was included. The new submission included these parts.

Fertilized eggs (140 eggs/level, 4 reps/level, 35 eggs/rep; <48 hrs old) of fathead minnow were exposed to 0 (negative and solvent controls), 0.93, 1.9, 3.8, 7.5 and 15 µg ai/L nominal concentrations. Time-weighted, mean-measured concentrations were <0.24-<0.42 (<LOQ; negative and solvent controls), 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L. The test system was maintained at 24 to 27 °C and a pH of 7.9-8.3. The 35-day EC_{50} and NOAEC values, based on survival, total length and wet weight, were >12 and 6.3 µg ai/L, respectively. The sub-lethal effects included reduced juvenile survival and inhibitions of total length and dry weight. The most sensitive end-points were percent survival, total length, and wet weight.

The hardness and alkalinity were five times the level that EPA allowed when the study was originally submitted, but these levels are now allowed, because they meet the OECD protocol.

This toxicity study is scientifically sound and satisfies the requirements of §72-4a for an early life toxicity study with *Pimephales promelas*. It is classified as **Acceptable.**

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Eggs, <48 Hrs
Test Type (Flow-through, Static, Static Renewal): Flow-Through

Percent Survival (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Total Length (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Wet Weight (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Endpoint(s) Affected: Percent Survival, Total Length and Wet Weight

I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in ASTM Standard Practice for Conducting Toxicity Tests on the Early Life Stages of Fishes, E-47.01; and U.S. EPA Proposed Recommended Bioassay Procedure for Egg and Dry Stages of Freshwater Fish (1972), unpublished manuscript, Environmental Research Laboratory, Duluth, MN. The following deviations from OPPTS 850.1400 were noted:

1. The physiochemical properties of the test material were not reported.
2. The test chambers were constructed with polyethylene, instead of a recommended glass or stainless steel material; while polyethylene could interact with the test material, measured concentrations throughout the study were consistent.
3. The age of the test organisms at test initiation (<48 Hrs) may have been higher than recommended in the guidance (2-24 Hrs).
4. The method of collection of fertilized eggs was not specified.
5. The amount of solvent used in the preparation of the stock solutions was not reported.
6. The reported hardness (225-275 mg/L as $CaCO_3$) and pH (7.9-8.3) of the dilution water were higher than recommended by EPA (40-48 mg/L as $CaCO_3$ and 7.2-7.6, respectively), but satisfy the OECD protocol.

These deviations do not affect the acceptability of the study.

COMPLIANCE: Signed and data Quality Assurance and GLP statements were provided. This study was conducted in compliance with the criteria promulgated by the Good Laboratory Practice regulations for Non-clinical Laboratory Studies (21 CFR, Part 58).

A. MATERIALS:

1. Test Material Supracide® (Methidathion)

Description: White Crystalline Solid

Lot No./Batch No. : FL830958 Methidathion (Lot No.)
Purity: 97.2%

Stability of compound under test conditions: Analytical verification of the test material in the dilution water was conducted on Days 0, 1, and then every 7 days thereafter. The time-weighted concentrations yielded recoveries of 73-85% of nominal.

Storage conditions of test chemicals: The test material was stored in the dark at 4°C.

Physicochemical properties of Methidathion.

Parameter	Values	Comments
Water solubility at 20EC	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

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

2. Test organism:

Species: Fathead Minnow (*Pimephales promelas*)
Age /embryonic stage at test initiation: <48 Hrs

EPA recommends fish embryos 2 to 24 hours old.

Method of collection of the fertilized eggs: Not Reported

Source: In-house Cultures

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 13-day flow-through toxicity test was conducted to determine the acute toxicity to fathead minnows. Nominal concentrations were 0.012, 0.025, 0.05, 0.1, and 0.2 mg ai/L. The 13-Day LC_{50} and NOAEC values were 0.15 and 0.012 mg ai/L, respectively.

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks / Criteria
Parental acclimation, if any		
Period:	Continuous	
Conditions (same as test or not):	Same as test	
Feeding (type, source, amount given,	Fed at least twice daily with a mixed diet of live newly hatched brine shrimp nauplii and ground commercial fish food (Rangen's®)	

Table 1: Experimental Parameters

Parameter	Details	Remarks Criteria
frequency): Health: (any mortality observed)	<5% during acclimation period	
Number of fertilized eggs/embryos in each treatment at test initiation	140 eggs/treatment; divided among 4 replicates, each containing 35 fertilized eggs.	*Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)*
Concentration of test material nominal: measured:	0 (negative and solvent controls), 0.93, 1.9, 3.8, 7.5 and 15 µg ai/L <0.24-<0.42 (<LOQ; negative and solvent controls), 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L	Measured concentrations were weighted for time. *A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.* *- Toxicant concentration should be measured in one tank at each toxicant level every week.* *- One concentration should adversely affect a life stage and one concentration should not affect any life stage. OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within ∀20% of the mean measured values.*
Solvent (type, percentage, if used)	Nanograde Acetone	The amount of solvent used was not reported. *The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD recommends that the solvent not have an 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:	 4 4 4	*Number of replicates should be 4 per concentration. A solvent control should be used in conjunction with a solubilizing agent.*
Test condition static renewal/flow-	Flow-Through	From April 6 to April 13, the diluter water flow was reduced due to a plugged filter, during which time the average flow replaced the test aquaria volume ~3 times every 24 hours.

Table 1: Experimental Parameters

Parameter	Details	Remarks Criteria
through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	Intermittent proportional diluter system described by Mount and Brungs ~7 volume replacements every 24 hours N/A	*Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water. 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 preferred;* *2) Aeration should not be used for mixing;* *3) The test solution should be completely mixed before introduction into the test system;* *4) Flow splitting accuracy should be within 10%.*
Aeration, if any	Water was aerated prior to introduction into the aquaria	*Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	35 Days	*Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.*
<u>Embryo cups, if used</u> type/material (glass/stainless steel): size: fill volume:	Polyethylene boxes with 40 mesh stainless steel screen fused to the sides 10 cm^2 Completely submersed in test vessels.	*Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
<u>Test vessel</u>		The water depth in the test vessels was 24 cm. *Recommended test vessel is all glass or glass with*

Table 1: Experimental Parameters

Parameter	Details	Remarks Criteria
type/material: (glass/stainless steel) size: fill volume:	Glass 23 x 15 x 30 cm 9.3 L	*stainless steel frame.*
Source of dilution water	Distilled water. No other details pertaining to the dilution water were provided.	*Source of dilution water should be 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:	 225-275 mg/L as $CaCO_3$ 7.9-8.3 7.3-9.3 mg/L 24-27°C 16L:8D N/A Ammonia- 0.12-0.35 mg/L Days 0, 1, 7, 14, 21, 28 and 35	The reported hardness (225-275 mg/L as $CaCO_3$) and pH (7.9-8.3) of the dilution water were higher than recommended (40-48 mg/L as $CaCO_3$ and 7.2-7.6, respectively). *Recommended hardness: 40-48 mg/L as $CaCO_3$;* *Recommended pH:* 7.2 to 7.6 *Dissolved Oxygen (DO) should be measured at each concentration at least once a week;* *Freshwater parameters in a control and one concentration should be analyzed once a week.* *Temperature depends upon test species and should not deviate by more than 2EC from appropriate temperature.* *OECD recommends that DO concentration be 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.*
<u>Post-hatch details</u> when the post-hatch period began: number of hatched eggs (alevins)/ treatment released to the test chamber: on what day, the alevins were released from the incubation cups to the	 Day 5 60 (15/rep) Day 9	*Percentage of embryos that produce live fry should be ≥ 50% in each control; percentage of hatch in any control embryo cup should not be more than 1.6 times that in another control cup.*

Table 1: Experimental Parameters

Parameter	Details	Remarks Criteria
test chamber:		
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	 Day 5 A mixed diet of live newly hatched brine shrimp nauplii and ground commercial fish food (Rangen's®) *ad libitum*. 3-4 times a day	
Stability of chemical in the test system	Stable, time-weighted recoveries were 73-85% of nominal and individual measured values ranged from 75-117% of the time-weighted concentrations.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	73-85% of nominal Days 0, 1, 7, 14, 21, 28 and 35 Not Reported 0.24-0.42 µg ai/L	
Positive control {if used, indicate the chemical and concentrations}	A 96-hour acute toxicity study was conducted with Antimycin A and was used as a reference.	This study was not conducted concurrently with the ELS study.
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	% egg hatch % post hatch survival length Weight	*Recommended parameters measured include:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and Juveniles:* *- Time to swim-up (if appropriate);* *- 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 (e.g., alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	 Days 0-5 Days 0-5 Daily N/A Day 35 N/A N/A	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Percent egg hatch [(no. of fry ÷ no. of eggs on day 0) x 100] was 78 and 94% in the negative and solvent controls, respectively, and 88, 91, 75, 89 and 75% in the measured 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L treatment groups, respectively. No significant differences were detected at any treatment levels relative to the controls.

Fry survival [(no. of fry on day 35 ÷ no. of fry introduced) x 100] was 91 and 98% in the negative and solvent controls, respectively, and 90, 90, 90, 90, 90 and 47% in the measured 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L treatment groups, respectively. The percent survival at the highest treatment level, measured 12 µg ai/L, was significantly reduced relative to the pooled controls. The resulting NOAEC and LOAEC values were 6.3 and 12 µg ai/L.

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

Treatment (µg ai/L) Measured (and Nominal) Concentrations	Egg hatched/embryo viability			Time to hatch			Juvenile-survival on day 35	
	No. of eggs at study initiation	hatch/embryo viability		day x1	day x2	day xn	No. dead	% mortality
		No.*	%					
Negative Control	140	109	78	N.R.	N.R.	N.R.	5	9
Solvent Control	140	132	94	N.R.	N.R.	N.R.	1	2
0.68 (0.93)	140	123	88	N.R.	N.R.	N.R.	6	10
1.6 (1.9)	140	127	91	N.R.	N.R.	N.R.	6	10
3.2 (3.8)	140	105	75	N.R.	N.R.	N.R.	6	10
6.3 (7.5)	140	125	89	N.R.	N.R.	N.R.	6	10
12 (15)	140	105	75	N.R.	N.R.	N.R.	32	53
NOAEC	12 µg ai/L			N.R.			6.3 µg ai/L	
EC_{50}	N.D.			N.D.			N.D.	
Positive control, if used mortality: EC_{50}: NOAEC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

*The number of eggs hatched was determined by the reviewer based on the number of eggs at initiation and the reported % hatch at each treatment level.
N.R.- Not Reported
N.D.- Not Determined
N/A- Not Applicable

Table 4: Effect of Supracide® on Growth of Juvenile Fish

Treatment (µg ai/L) Measured (and Nominal) Concentrations	Swim-up			Growth -length (mm ±S.D.)	Growth-wet weight (mg ±S.D.)
	day x1	day x2	day xn		
Negative Control	N.D.	N.D.	N.D.	16 (±2.0)	76 (±30)
Solvent Control	N.D.	N.D.	N.D.	16 (±2.0)	74 (±31)
0.68 (0.93)	N.D.	N.D.	N.D.	15 (±22)	73 (±31)
1.6 (1.9)	N.D.	N.D.	N.D.	16 (±2.4)	76 (±32)
3.2 (3.8)	N.D.	N.D.	N.D.	16 (±2.6)	74 (±37)
6.3 (7.5)	N.D.	N.D.	N.D.	15 (±1.8)	70 (±26)
12 (15)	N.D.	N.D.	N.D.	12 (±2.2)*	38 (±21)*
NOAEC	N.D.			6.3 µg ai/L	6.3 µg ai/L
LOAEC	N.D.			12 µg ai/L	12 µg ai/L
EC_{50}	N.D.			N.D.	N.D.
Positive control, if used mortality: EC_{50}: NOAEC	N/A	N/A	N/A	N/A	N/A

N.D.- Not Determined; N/A- Not Applicable; *- Significantly different (α=0.05) from the control using one-way ANOVA and Fisher's protected Least Significant Difference.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Clinical signs of toxicity during the definitive toxicity test were not provided in the raw data. Time to hatch and time to swim-up were not evaluated; however, the growth period began on Day 5 with greater than 95% hatch.

Total length and wet weight were the most sensitive endpoints. There were statistically significant reductions detected for both endpoints at the highest measured treatment level (12 µg ai/L) relative to the pooled control. Total length averaged 16 mm in both controls, and 15, 16, 16, 15 and 12 mm in the measured 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L treatment groups, respectively. Wet weight averaged 76 and 74 mg in the negative and solvent controls, and 73, 76, 74, 70 and 38 mg in the measured 0.68, 1.6, 3.2, 6.3, and 12 µg ai/L treatment groups, respectively. The NOAEC and LOAEC for both endpoints were 6.3 and 12 µg ai/L, respectively.

Table 5: Sub-lethal Effect of Supracide® on Fathead Minnow.

Treatment (µg ai/L) Measured (and Nominal) Concentrations	% Deformed larvae	Behavioral effects (specify)	Behavioral effects (specify)	Toxicity symptoms (specify)	Toxicity symptoms (specify)
Negative Control	N.R.	N.R.	N.R.	N.R.	N.R.
Solvent Control	N.R.	N.R.	N.R.	N.R.	N.R.
0.68 (0.93)	N.R.	N.R.	N.R.	N.R.	N.R.
1.6 (1.9)	N.R.	N.R.	N.R.	N.R.	N.R.
3.2 (3.8)	N.R.	N.R.	N.R.	N.R.	N.R.
6.3 (7.5)	N.R.	N.R.	N.R.	N.R.	N.R.
12 (15)	N.R.	N.R.	N.R.	N.R.	N.R.
NOAEC	N.R.				
LOAEC	N.R.				
Positive control, if used % sublethal effect: NOAEC:	N/A	N/A	N/A	N/A	N/A

N.R.- Not Reported
N/A- Not Applicable

C. REPORTED STATISTICS:

The design of the study was a randomized complete block design. Measured parameters of standard length and wet weight in the quadruplicate exposure aquaria were analyzed using a two-way ANOVA with an inter-action model to determine whether any interaction was present between the two factors (concentration and block). The data were then analyzed to determine whether there was any significant effect due to block, i.e. replication (4). If the analysis indicated no significant interaction, data were pooled for further analysis.

Comparison analysis between the water control and treatment levels were carried out using hatchability, survival, standard length, and wet weight. The data were compared using the overall one-way ANOVA to determine if a significant difference (α=0.05) existed between the control and treatment levels. When treatment effects were indicated following a significant F-test of the mean square ratios, a multiple means comparison test, Least Significant Difference (LSD), was used to determine which exposure levels differed from the control values. All toxicity values were based on the mean-measured concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Fry survival on Day 35 (30 days post-hatch), total length (30 days post-hatch) and wet weight (30 days post-hatch) were analyzed for significant differences.

For fry, survival and wet-weight, the negative and solvent controls were analyzed using a Student's t-test and no significant differences were detected. No variability existed between the controls for total length.

Data from all three endpoints were analyzed for normality using Chi-square and Shapiro Wilks tests and for homogeneity of variance using Hartley and Bartlett's tests. Fry survival and wet weight met the assumptions of ANOVA and were therefore analyzed using the Bonferroni t-test and Williams test. Total length did not meet the assumptions of ANOVA and was analyzed using the non-parametric Kruskal-Wallis test. The raw data for replicate C of the highest treatment level (measured 12 µg ai/L) was excluded from all analyses, because it was not clear to the reviewer how many fish were observed and measured. All toxicity values were determined using the time-weighted averages. Raw data were not provided for mean percent hatch; therefore, the reviewer was unable to analyze this endpoint.

Percent Survival (Day 35):
EC_{50}: >12 µg ai/L (95% C.I.: N/A) Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L LOAEC: 12 µg ai/L

Total Length (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L LOAEC: 12 µg ai/L

Wet Weight (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L LOAEC: 12 µg ai/L

E. STUDY DEFICIENCIES: There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results were obtained using the time-weighted measured concentrations while those of the study authors were based on the mean-measured concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The time-weighted measured concentrations were calculated by the reviewer using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C_{TWA} is the time-weighted average concentration,
C_j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j
(e.g., t_0 = 0 hours (test initiation), t_1 =24 hours, t_2 =96 hours)

The study authors reported that replicate D of the solvent control was not included in the statistical analyses due to non-toxicant related mortality.

The results from a period screening analysis of the dilution water indicated the presence of the following elements: lead (0.017 ppm), mercury (0.0008 ppm) and zinc (0.001 ppm).

A method validation study for the analysis of the test material in the dilution water was conducted using spikes of 0.10, 1.00, 10.0, 100, and 1000 µg ai/L. The overall mean recovery (±S.D.) was 93.7% (±10.3).

The in-life portion of the definitive toxicity test was conducted from March 21 to April 25, 1984.

G. CONCLUSIONS:

The study is scientifically sound and fulfils the guideline requirements. It is classified as ACCEPTABLE. Fry survival (Day 35), total length (Day 35) and wet-weight (Day 35) were equally sensitive to the test material with NOAEC, LOAEC, and EC_{50} values of 6.3, 12 and >12 µg ai/L, respectively.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN	= 91.5000	CALCULATED t VALUE	= -1.4916
GRP2 (BLANK CRTL) MEAN	= 98.2500	DEGREES OF FREEDOM	= 6
DIFFERENCE IN MEANS	= -6.7500		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.541	5.566	8.786	5.566	1.541
OBSERVED	0	7	6	10	0

Calculated Chi-Square goodness of fit test statistic = 7.8671
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1935.417

W = 0.924

Critical W (P = 0.05) (n = 23) = 0.914
Critical W (P = 0.01) (n = 23) = 0.881

Data PASS normality test at P=0.01 level. Continue analysis.

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 20.92
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.83
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 7.59
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.83
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	4815.192	963.038	8.459
Within (Error)	17	1935.417	113.848	
Total	22	6750.609		

Critical F value = 2.81 (0.05,5,17)
Since F > Critical F REJECT Ho:All groups equal

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs 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	neg control	91.500	91.500		
2	0.68	88.250	88.250	0.431	
3	1.6	87.500	87.500	0.530	
4	3.2	90.000	90.000	0.199	
5	6.3	90.000	90.000	0.199	
6	12	46.667	46.667	5.501	*

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=17,5)

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs 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	neg control	4			
2	0.68	4	19.368	21.2	3.250
3	1.6	4	19.368	21.2	4.000
4	3.2	4	19.368	21.2	1.500
5	6.3	4	19.368	21.2	1.500
6	12	3	20.919	22.9	44.833

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	91.500	91.500	91.500
2	0.68	4	88.250	88.250	88.938
3	1.6	4	87.500	87.500	88.938
4	3.2	4	90.000	90.000	88.938
5	6.3	4	90.000	90.000	88.938
6	12	3	46.667	46.667	46.667

Fry survival (%), 30 days post-hatch; ug ai/L
File: 2701fs 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
neg control	91.500				
0.68	88.938	0.340		1.74	k= 1, v=17
1.6	88.938	0.340		1.82	k= 2, v=17
3.2	88.938	0.340		1.85	k= 3, v=17
6.3	88.938	0.340		1.87	k= 4, v=17
12	46.667	5.501	*	1.87	k= 5, v=17

s = 10.670
Note: df used for table values are approximate when v > 20.

Total length (mm), 30 days post hatch; ug ai/L
File: 2701tl Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.541	5.566	8.786	5.566	1.541
OBSERVED	0	9	8	6	0

Calculated Chi-Square goodness of fit test statistic = 5.3048
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Total length (mm), 30 days post hatch; ug ai/L
File: 2701tl Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 6.167

W = 0.941

Critical W (P = 0.05) (n = 23) = 0.914
Critical W (P = 0.01) (n = 23) = 0.881

Data PASS normality test at P=0.01 level. Continue analysis.

Total length (mm), 30 days post hatch; ug ai/L
File: 2701tl Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Total length (mm), 30 days post hatch; ug ai/L
File: 2701tl Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	16.000	16.000	72.000
2	0.68	15.500	15.500	54.000
3	1.6	15.500	15.500	54.000
4	3.2	15.750	15.750	59.000
5	6.3	14.750	14.750	31.000
6	12	12.333	12.333	6.000

Calculated H Value = 13.930 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Total length (mm), 30 days post hatch; ug ai/L
File: 2701tl Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 2 3 4 1
6	12	12.333	12.333	\
5	6.3	14.750	14.750	. \
2	0.68	15.500	15.500	. . \
3	1.6	15.500	15.500	. . . \
4	3.2	15.750	15.750	. . . . \
1	neg control	16.000	16.000	* . . . . \

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* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	5.9	4.2	8.4	0.072	0.71
EC10	8.0	6.5	9.9	0.045	0.81
EC25	13.	11.	15.	0.028	0.87
EC50	23.	16.	32.	0.073	0.70

Slope = 2.81 Std.Err. = 0.651

Goodness of fit: p = 0.56 based on DF= 3.0 17.

2701TL : Total length (mm), 30 days post hatch; ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	16.0	15.7	0.289	100.	0.00
0.680	4.00	15.5	15.7	-0.211	100.	0.000893
1.60	4.00	15.5	15.7	-0.202	99.9	0.0586
3.20	4.00	15.8	15.6	0.168	99.2	0.820
6.30	4.00	14.8	14.8	-0.0524	94.2	5.78
12.0	3.00	12.3	12.3	0.0110	78.4	21.6

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	76.5000	CALCULATED t VALUE =	0.8839
GRP2 (BLANK CRTL) MEAN =	74.2500	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	2.2500		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.541	5.566	8.786	5.566	1.541
OBSERVED	0	8	8	7	0

Calculated Chi-Square goodness of fit test statistic = 4.5861
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1150.500

W = 0.927

Critical W (P = 0.05) (n = 23) = 0.914
Critical W (P = 0.01) (n = 23) = 0.881

Data PASS normality test at P=0.01 level. Continue analysis.

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 5.02
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.83
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 2.29
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.83
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2248.457	449.691	6.645

Within (Error)	17	1150.500	67.676
Total	22	3398.957	

Critical F value = 2.81 (0.05,5,17)
Since F > Critical F REJECT Ho:All groups equal

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww 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	neg control	76.500	76.500		
2	0.68	74.000	74.000	0.430	
3	1.6	77.000	77.000	-0.086	
4	3.2	75.250	75.250	0.215	
5	6.3	70.750	70.750	0.988	
6	12	46.000	46.000	4.854	*

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=17,5)

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww 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	neg control	4			
2	0.68	4	14.932	19.5	2.500
3	1.6	4	14.932	19.5	-0.500
4	3.2	4	14.932	19.5	1.250
5	6.3	4	14.932	19.5	5.750
6	12	3	16.129	21.1	30.500

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	76.500	76.500	76.500
2	0.68	4	74.000	74.000	75.500
3	1.6	4	77.000	77.000	75.500
4	3.2	4	75.250	75.250	75.250
5	6.3	4	70.750	70.750	70.750
6	12	3	46.000	46.000	46.000

Wet Weight (mg), 30 days post-hatch; ug ai/L
File: 2701ww 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
neg control	76.500				
0.68	75.500	0.172		1.74	k= 1, v=17
1.6	75.500	0.172		1.82	k= 2, v=17
3.2	75.250	0.215		1.85	k= 3, v=17
6.3	70.750	0.988		1.87	k= 4, v=17
12	46.000	4.854	*	1.87	k= 5, v=17

s = 8.227
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	5.9	3.4	10.	0.11	0.58
EC10	7.1	4.7	11.	0.085	0.67
EC25	9.7	8.0	12.	0.041	0.82
EC50	14.	11.	17.	0.040	0.83

Slope = 4.40 Std.Err. = 1.60

Goodness of fit: p = 0.96 based on DF= 3.0 17.

2701WW : Wet Weight (mg), 30 days post-hatch; ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	76.5	75.7	0.756	100.	0.00
0.680	4.00	74.0	75.7	-1.74	100.	4.31e-07
1.60	4.00	77.0	75.7	1.26	100.	0.00188
3.20	4.00	75.3	75.5	-0.299	99.7	0.258
6.30	4.00	70.8	70.7	0.0332	93.4	6.64
12.0	3.00	46.0	46.0	-0.00483	60.7	39.3

!!!Warning: EC50 not bracketed by doses evaluated.