

Data Evaluation Report on the Toxicity of Ziram to Fathead Minnow (*Primephales promelas*), Early Life Cycle

PMRA Submission Number {.....}

EPA MRID Number 468931-04

Data Requirement: PMRA Data Code {.....}
EPA DP Barcode D323417
OECD Data Point {.....}
EPA MRID 468931-04
EPA Guideline 850.1400 (OPP §72-4a)

Test material: [14C]Ziram Radiochemical Purity: 97%
Unlabeled Ziram Technical Purity: 98.2%

Common name Ziram
Chemical name: IUPAC: Zinc bis(dimethyldithiocarbamate)
CAS name: (T-4)-bis(Dimethylcarbomodithioato-κS,κS')zinc
CAS No.: 137-30-4
Synonyms: Ziram PHYTO

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Date: 11/13/06

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Date: {.....}

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CITATION: Palmer, S.J., T.Z. Kendall, and H.O. Krueger. 2006. Ziram: An Early Life-Stage Toxicity Test with the Fathead Minnow (Pimephales promelas). Unpublished study performed by Wildlife International., Ltd., Easton, MD. Laboratory Project No. 602A-103A. Study submitted by The Ziram Task Force, c/o Cerexagri, Inc., King of Prussia, PA. Study initiated February 8, 2006 and submitted July 18, 2006.

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.

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EXECUTIVE SUMMARY:

The 33-day chronic toxicity of ziram to the early life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs/embryos (80/level, <24 hours old) of fathead minnow were exposed to a mixture of radiolabeled plus unlabeled ziram at nominal concentrations of 0 (negative and solvent controls), 47, 94, 188, 375, and 750 µg ai/L. Mean-measured concentrations were <LOQ, 48, 101, 195, 393 and 750 µg total residues/L. TWA concentrations were <7.26 (<LOQ, controls), 49, 101, 196, 395, and 750 µg total residues/L, respectively. The test system was maintained at 24.0-25.8 °C and a pH of 8.0-8.1. The 33-day EC₅₀ was 218 µg ai/L for post-hatch survival and 590 µg total residues/L for hatching success. NOAEC and LOAEC values were 101 and 196 µg total residues/L, respectively, based on post-hatch larval survival.

Hatching occurred during days 3 to 5 at all levels, with no treatment-related effect observed. Hatching success, however, was statistically-reduced at the 750 µg total residues/L level compared to the negative control (10 versus 98%, respectively). All hatched larvae from the 750 µg total residues/L level died within 1 day post-hatch, and all hatched larvae from the 395 µg total residues/L level died within 21 days post-hatch. Organisms from these two levels were weak and/or small prior to death. Post-hatch larval survival was the most sensitive endpoint, with statistically-significant reductions from the negative control observed at the ≥195 µg total residues/L levels. On Day 33 (28 days post-hatch), larvae survival averaged 94% in the negative control group, compared to 88, 95, 97, and 72% in the solvent control and mean-measured 48, 101, and 195 µg total residues/L groups, respectively. No treatment-related effect on terminal growth was observed at up to 195 µg total residues/L.

This study is scientifically sound and is classified as ACCEPTABLE for an early life toxicity study with fathead minnow.

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Embryos, <24 hours old

Test Type (Flow-through, Static, Static Renewal): Flow-through

LOAEC: 195 µg total residues/L

Post-hatch Survival NOAEC: 101 µg total residues/L

Endpoint(s) Affected: hatching success, post-hatch clinical signs of toxicity, and post-hatch survival

Most Sensitive Endpoint(s): post-hatch survival

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I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OECD Guidelines for Testing of Chemicals, Guideline 210, *Fish Early-Life Stage Toxicity Test*; U.S. EPA Series 850 – Ecological Effects Test Guidelines (draft), OPPTS No. 850.1400 *Fish Early Life-Stage Test*; ASTM Standard E1241-88, *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish*; and U.S. Environmental Protection Agency Standard Evaluation Procedure, *Fish Early Life-Stage Test*. Deviations from OPPTS No. 850.1400 included:

Test samples were analyzed only for total radioactive residues using LSC. The radioactivity was not further characterized; therefore, the stability of ziram under actual use conditions was not verified.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in accordance with GLP Standards as published in 40 CFR Part 160 with the following exception: periodic analysis of well water for potential contaminants.

A. MATERIALS:

1. Test Material Ziram Technical and ¹⁴C-Ziram

Description: Solids

Lot No./Batch No. : G4A0051877 (non-radiolabeled) and XV/36 (radiolabeled)

Purity: 98.2% (non-radiolabeled) and 97% (radiolabeled)

Stability of compound under test conditions: Unverified. Test samples collected on days 0, 7, 14, 21, 28, and 34 were analyzed for total radioactivity using LSC. All results were within 20% among replicate measurements; however, the radioactivity was not further characterized.

Storage conditions of test chemicals: Frozen

Physicochemical properties of Ziram.

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)

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2. Test organism:

Species:

Fathead minnow (*Pimephales promelas*) [EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.]

Age /embryonic stage at test initiation:

Embryos, <24 hours old [EPA recommends fish embryos 2 to 24 hours old.]

Method of collection of the fertilized eggs:

Embryos were removed from spawning substrates and examined under a dissecting microscope to select healthy, viable specimens at approximately the same stage of development.

Source:

Chesapeake Cultures Inc., Hayes, VA

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The concentrations were selected in consultation with the Sponsor, and were based upon the results of exploratory range-finding data (not further specified).

b. Definitive study

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)	N/A	Embryos collected for use in the test were purchased, and were from eight individual spawns.
Number of fertilized eggs/embryos in each treatment at test initiation	80 embryos/treatment level, divided into 20 embryos/cup, 1 cup/aquarium, and 4 replicate aquaria/treatment.	Fish were not thinned following hatching. ----- 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)

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Parameter	Details	Remarks
		Criteria
<p><u>Concentration of test material</u> nominal:</p> <p>mean measured:</p> <p>TWA (reviewer-calculated):</p>	<p>0 (negative and solvent controls), 47, 94, 188, 375, and 750 µg ai/L</p> <p><7.26 (<LOQ, controls), 48, 101, 195, 393, and 750 µg total residues/L, respectively</p> <p><7.26 (<LOQ, controls), 49, 101, 196, 395, and 750 µg total residues/L, respectively</p>	<p>Total radioactive concentrations were determined using LSC at 0, 7, 14, 21, 28, and 33 days. All measured concentrations were within 20% among replicates. The radioactivity was not characterized to determine what percentage was parent material.</p> <hr/> <p><i>A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.</i></p> <ul style="list-style-type: none"> - 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. <p><i>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.</i></p>
<p>Solvent (type, percentage, if used)</p>	<p>Dimethyl formamide, 0.1 ml/L</p>	<hr/> <p><i>The solvent should not exceed 0.1 ml/L in a flow-through system.</i></p> <p><i>Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</i></p> <p><i>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.</i></p>
<p><u>Number of replicates</u> control: solvent control: treated ones:</p>	<p>4 4 4/level</p>	<hr/> <p><i>Number of replicates should be 4 per concentration.</i></p> <p><i>A solvent control should be used in conjunction with a solubilizing agent.</i></p>

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Parameter	Details	Remarks
		Criteria
<p><u>Test condition</u></p> <p>static renewal/flow-through:</p> <p>type of dilution system for flow through method:</p> <p>flow rate:</p> <p>renewal rate for static renewal:</p>	<p>Flow-through</p> <p>Continuous-flow serial diluter</p> <p>Approx. 10 volume additions per day</p> <p>N/A</p>	<p>The syringe pump and rotameters were calibrated prior to test initiation and verified at approximately weekly intervals during the test.</p> <hr/> <p><i>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.</i></p> <p><i>Toxicant Mixing:</i></p> <p>1) Mixing chamber is preferred;</p> <p>2) Aeration should not be used for mixing;</p> <p>3) The test solution should be completely mixed before introduction into the test system;</p> <p>4) Flow splitting accuracy should be within 10%.</p>
<p>Aeration, if any</p>	<p>None reported.</p>	<hr/> <p><i>Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</i></p>
<p>Duration of the test</p>	<p>33 days (28-days post-hatch)</p>	<hr/> <p><i>Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.</i></p>

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Parameter	Details	Remarks
		Criteria
<p><u>Embryo cups, if used</u></p> <p>type/material (glass/stainless steel):</p> <p>size:</p> <p>fill volume:</p>	<p>Glass cylinders with 425-μm nylon screen mesh attached to the bottom with silicone sealant</p> <p>Approx. 50 mm in diameter</p> <p>Not reported</p>	<p>The embryo cages were oscillated slowly (approx. 2 rpm) to assure an adequate flow of media around the embryos.</p> <hr/> <p><i>Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</i></p>
<p><u>Test vessel</u></p> <p>type/material: (glass/stainless steel)</p> <p>size:</p> <p>fill volume:</p>	<p>Glass</p> <p>9 L</p> <p>7 L (15-cm depth)</p>	<hr/> <p><i>Recommended test vessel is all glass or glass with stainless steel frame.</i></p>
<p>Source of dilution water</p>	<p>Moderately-hard freshwater was obtained from a well approximately 40 m deep located on site. The well water was passed through a sand filter, aerated, filtered again (0.45 μm), and UV-sterilized prior to use.</p> <p>Results from the weekly water analyses for the 4 weeks immediately preceding the study were as follows: specific conductance of 300-310 μmhos/cm, hardness of 128-140 mg/L as CaCO₃, alkalinity of 180-184 mg/L as CaCO₃, and pH of 7.9-8.1.</p>	<p>Results of periodic analysis for pesticides, organics, and metals were also provided (from water collected on 12/15/05). All pesticides and organics were below the LOD. The following metals were present: calcium at 33.1 mg/L, chloride at 2.7 mg/L, fluoride at 0.56 mg/L, magnesium at 13.3 mg/L, potassium at 7.65 mg/L, and sodium at 19.1.</p> <hr/> <p><i>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.</i></p>

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Parameter	Details	Remarks
		Criteria
<p><u>Water parameters</u> hardness:</p> <p>pH:</p> <p>dissolved oxygen:</p> <p>temperature (s) (record all the temperatures used for different life stages):</p> <p>photoperiod:</p> <p>salinity (for marine or estuarine species):</p> <p>other measurements:</p> <p>interval of water quality measurements:</p>	<p>132-144 mg/L as CaCO₃</p> <p>8.0-8.1</p> <p>≥6.4 mg/L (≥78% saturation)</p> <p>24.0-25.8°C (maintained constant throughout study)</p> <p>16 hours light/8 hours dark, with 30-minute transition periods</p> <p>N/A</p> <p>Specific conductance of 335-365 µmhos/cm; and alkalinity of 182-186 mg/L as CaCO₃</p> <p>Temperature was measured in each chamber at least weekly and in one negative control replicate continuously. DO was measured in alternating replicates of each level daily during the first 7 days and weekly thereafter. pH was measured in alternating replicates of each level at least weekly. Hardness, alkalinity, and specific conductance were measured in alternating replicates of the negative control and 750 µg ai/L levels at least weekly.</p>	<p>Light intensity at test initiation was 194 lux over one representative test chamber.</p> <hr/> <p><i>Recommended hardness: 40-48 mg/L as CaCO₃;</i> <i>Recommended pH: 7.2 to 7.6</i> <i>Dissolved Oxygen (DO) should be measured at each concentration at least once a week;</i> <i>Freshwater parameters in a control and one concentration should be analyzed once a week.</i> <i>Temperature depends upon test species and should not deviate by more than 2EC from appropriate temperature.</i> <i>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.</i> <i>Temperature should be measured continuously.</i></p>
<p><u>Post-hatch details</u> when the post-hatch period began:</p> <p>number of hatched eggs (alevins)/ treatment released to the test chamber:</p> <p>on what day, the alevins were released from the incubation cups to the test chamber:</p>	<p>Day 5, when hatching was >90% complete in the negative control chambers.</p> <p>All hatched larvae were released.</p> <p>Day 5</p>	<p>Survival ranged from 95-100% in the negative control replicates.</p> <hr/> <p><i>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.</i></p>

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Parameter	Details	Remarks
		Criteria
<u>Post-hatch Feeding</u> start date: type/source of feed: amount given: frequency of feeding:	Day 5 Live brine shrimp nauplii (<i>Artemia sp.</i>) Not reported Three times daily during the first 7 days post-hatch, and at least two times daily thereafter. Fish were not fed during the final 48 hours of the test.	To ensure that the feeding rate per fish remained constant, rations were adjusted each week to account for losses due to mortality.
Stability of chemical in the test system	Measurement of total radioactive residues (LSC) indicated relatively constant measured concentrations (within 20% among replicate measurements). However, the stability of ziram was not verified.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	93.3-113% of nominal Days 0, 7, 14, 21, 28, and 33 Not reported 7.26 µg total residues/L	Based on LSC analysis of test samples.
Positive control {if used, indicate the chemical and concentrations}	N/A	
<u>Fertilization success study, if any</u> number of eggs used: on what day the eggs were removed to check the embryonic development:	N/A	
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observations

Parameters	Details	Remarks
		Criteria
Parameters measured including the sublethal effects/toxicity symptoms	<ul style="list-style-type: none"> - Embryo survival - Time to hatch - Hatching success - Larval survival - Measurement of growth (total length, wet weight, and dry weight) - Clinical signs of toxicity or abnormal behavior 	<p><i>Recommended parameters measured include:</i></p> <ul style="list-style-type: none"> - 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.
<p>Observation intervals/dates for:</p> <p>egg mortality: no. of eggs hatched: mortality of fry (e.g., alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects</p>	<p>Daily Daily Daily N/A Day 33 Not determined Daily</p>	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

On Day 5, hatching success averaged 98 and 100% in the negative and solvent control groups, respectively, compared to 98, 99, 95, 96, and 10% in the mean-measured 48, 101, 195, 393, and 750 µg total residues/L levels, respectively. The difference at the 750 µg total residues/L level was statistically-reduced compared to the pooled control ($p \leq 0.05$). The NOAEC for hatching success was 393 µg total residues/L.

On Day 33 (28 days post-hatch), larvae survival averaged 94% in the negative control group, compared to 88, 95, 97, and 72% in the solvent control and mean-measured 48, 101, and 195 µg total residues/L groups, respectively. The difference at the 195 µg total residues/L level was statistically-reduced compared to the pooled control ($p \leq 0.05$). No larvae survived at the 393 and 750 µg total residues/L levels. The NOAEC for post-hatch survival was 101 µg total residues/L.

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Table 3: Effect of Ziram on Egg Hatching and Survival at Different Life Stages of Fish.

Treatment Measured, μg total residues/L (and nominal, $\mu\text{g ai/L}$) concentrations	Egg hatched/embryo viability			Time to hatch/ No. hatched			Juvenile-survival on day 33	
	No. of eggs at study initiation	hatch/embryo viability		Day 3	Day 4	Day 5	No. dead	% mortality
		No.	%					
Control (dilution water only)	80	78	98	3	50	78	5	6
Solvent control	80	80	100	0	29	80	10	12
48 (47)	80	78	98	1	14	78	4	5
101 (94)	80	79	99	0	55	79	2	3
195 (188)	80	76	95	0	26	76	21	28*
393 (375)	80	77	96	0	65	77	77	100*
750 (750)	80	8	10*	6	7	8	8	100*
NOAEC	393 μg total residues/L		750 μg total residues/L			101 μg total residues/L		
EC ₅₀	NR		NR			NR		
Positive control, if used	N/A		N/A			N/A		
mortality: EC ₅₀ : NOAEC								

NR – Not reported

* Statistically-significant difference from pooled control using Fisher's Exact test ($p \leq 0.05$).

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Table 4: Effect of Ziram on Growth of Juvenile Fish.

Treatment Measured, μg total residues/L (and nominal, μg ai/L) concentrations	Swim-up ^(a)			Growth - length (mm)	Growth-wet weight (mg)	Growth-dry weight (mg)
	day x1	day x2	day xn			
Control (dilution water only)	N/A	N/A	N/A	21.5	76.0	13.7
Solvent control	N/A	N/A	N/A	21.9	82.2	15.2
48 (47)	N/A	N/A	N/A	21.8	83.2	15.1
101 (94)	N/A	N/A	N/A	21.0*	72.8	12.7*
195 (188)	N/A	N/A	N/A	21.6	83.5	14.4
393 (375)	N/A	N/A	N/A	---	---	---
750 (750)	N/A	N/A	N/A	---	---	---
NOAEC	N/A	N/A	N/A	195 μg total residues/L	195 μg total residues/L	195 μg total residues/L
LOAEC	N/A	N/A	N/A	>195 μg total residues/L	>195 μg total residues/L	>195 μg total residues/L
EC ₅₀	N/A	N/A	N/A	NR	NR	NR
Positive control, if used	N/A	N/A	N/A	N/A	N/A	N/A
mortality: EC ₅₀ : NOAEC						

^(a) Swim-up is generally not applicable for this species.

*Statistically-significant from pooled control using Bonferroni's test ($p \leq 0.05$), but was not considered to be treatment-related due to lack of concentration-dependent response.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Daily observations of the fathead minnow embryos indicated that there were no apparent differences in time to hatch between the control groups and the ziram treatment groups (visually assessed). Embryos hatched on days 3, 4, and 5, and were released on day 5. The NOAEC for time to hatch was 750 μg total residues/L.

In general, the majority of fish in the control groups and in the 48, 101, and 195 μg total residues/L treatment groups appeared normal throughout the test. There were a few observations of organisms that appeared smaller, weak or lethargic, were swimming erratically, or had a curved spine; however, these observations were few and did not occur in a concentration-responsive pattern. Observations of weak and/or smaller fish were observed in the 393 and 750 μg total residues/L levels prior to 100% mortality. The NOAEC for clinical signs of toxicity was 195 μg total residues/L.

Growth was evaluated on day 33 in surviving fish by measuring the total length, wet weight, and dry weight. Although there was a statistically-significant effect on survival in the 195 μg total residues/L treatment group,

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the study authors reported that there was a sufficient number of fish that survived to make valid statistical analyses of growth. Both total length and dry weight were statistically-reduced compared to the pooled control ($p < 0.05$) at the 101 μg total residues/L level; however, a similar reduction was not observed at the 195 μg total residues/L level. Therefore, the small difference observed at the 101 $\mu\text{g}/\text{L}$ level was not considered to be biologically meaningful, and the NOAEC was considered to be 195 μg total residues/L.

Table 5: Sub-lethal Effects of Ziram on Fathead Minnow^(a).

Treatment Measured, μg total residues/L (and nominal, $\mu\text{g ai/L}$) concentrations	Weak, %	Crooked spine/tail, %	Small, %	Erratic Swimming, %	Lethargic, %
Control (dilution water only)	3	1	0	0	0
Solvent control	10	1	3	1	1
48 (47)	4	1	1	1	1
101 (94)	1	0	1	0	0
195 (188)	1	1	2	0	0
393 (375)	38	0	38	0	0
750 (750)	100	0	0	0	0
NOAEC	195 μg total residues/L				
LOAEC	393 μg total residues/L				
Positive control, if used % sublethal effect: NOAEC:	N/A	N/A	N/A	N/A	N/A

^(a) The maximum percentage of surviving fish exhibiting effect (reviewer-calculated).

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C. REPORTED STATISTICS:

Data that were statistically analyzed included 1) hatching success, 2) larval survival, 3) the mean total length of surviving fish at study termination, 4) the mean wet weight of surviving fish at study termination, and 5) the mean dry weight of surviving fish at study termination. The time to hatch was visually evaluated.

For all endpoints, responses from the negative and solvent control groups were compared using a t-test. No significant differences were observed, and the controls were pooled for all subsequent analyses. Hatching success and larval survival data were analyzed using Chi-square and Fisher's Exact test to identify treatment groups that showed a statistically significant difference from pooled controls ($p \leq 0.05$). Growth data were checked for normality using Chi-square, and for homogeneity of variance using Levene's test. The data passed these assumptions, and were subsequently analyzed using analysis of variance (ANOVA) and Bonferroni's t-test to identify treatments that were significantly different from the pooled control ($p \leq 0.05$).

The NOAEC and LOAEC were based on significance data. All analyses were performed using TOXSTAT or SAS software programs and mean-measured concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Hatching success, post-hatch survival, length, wet and dry weight were statistically analyzed. Data were analyzed using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett's tests for homogeneity of variances. Data satisfied these assumptions and NOAEC values were determined using ANOVA, followed by Dunnett's test. For all endpoints, the negative control was compared to the solvent control using a Student's t-test; no differences were detected and the negative control group was used for all comparisons to the treatment data. These analyses were conducted using TOXSTAT statistical software. The EC_{50} value for post-hatch survival was determined using the Probit method via Toxanal statistical software, while the EC_{50} value for hatching success was determined using the Probit method via Nuthatch.

EC_{50} : 218 μg total residues/L	95% C.I.: Not determinable
Probit Slope: 4.44	95% C.I.: -4.37-13.3
NOAEC: 101 μg total residues/L	
LOAEC: 195 μg total residues/L	

E. STUDY DEFICIENCIES:

This study is scientifically sound and provides useful data on the early life-stage toxicity of ziram to fathead minnow. However, as test samples were only analyzed for total radioactive residues, the stability of ziram under test conditions was not determined.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study authors'. The reviewer additionally attempted to estimate the EC_{50} for hatching success and survival. The estimate for survival was associated with an indefinable 95% confidence interval, so this result should be interpreted with caution if used quantitatively. Reviewer-calculated TWA concentrations are also provided (see Appendix 2 of this DER).

All test solutions appeared clear and colorless in the test chambers at test initiation and termination. A white precipitate was present in the diluter mixing chambers for the 375 and 750 $\mu\text{g}/\text{L}$ nominal treatments during the test.

Biomass loading at the end of the test was 0.021 g fish/L/day (instantaneous 0.22 g fish/L), based on the negative control group.

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The specific activity of the radiolabeled ziram was 11.3 MBq/mg.

In-life dates were May 4 – June 6, 2006.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. Based on a treatment-related effect upon larval survival (the most sensitive endpoint), the NOAEC and LOAEC are 101 and 195 µg total residues/L, respectively. Hatching success was also affected by exposure at the highest treatment level, and clinical signs of toxicity were observed in hatched larvae prior to death at the 393 and 750 µg total residues/L levels.

LOAEC: 195 µg total residues/L

Post-hatch Survival NOAEC: 101 µg total residues/L

Endpoint(s) Affected: hatching success, post-hatch clinical signs of toxicity, and post-hatch survival
Most Sensitive Endpoint(s): post-hatch survival

III. REFERENCES:

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APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Survival EC50

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	3.93981	39.07091	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.439874
 95 PERCENT CONFIDENCE LIMITS = -4.372812 AND 13.25256

LC50 = 217.5162
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 112.5764
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

survival (%)
 File: 3104sn Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	93.5000	CALCULATED t VALUE =	0.7735
GRP2 (BLANK CRTL) MEAN =	87.5000	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	6.0000		

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

survival (%)
 File: 3104sn 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	1.072	3.872	6.112	3.872	1.072
OBSERVED	0	5	4	7	0

Calculated Chi-Square goodness of fit test statistic = 5.7294
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

survival (%)

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File: 3104sn Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1555.750

W = 0.899

Critical W (P = 0.05) (n = 16) = 0.887

Critical W (P = 0.01) (n = 16) = 0.844

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

survival (%)

File: 3104sn Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 46.92

Closest, conservative, Table H statistic = 120.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 4, df (# reps-1) = 3

Actual values ==> R (# groups) = 4, df (# avg reps-1) = 3.00

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).

survival (%)

File: 3104sn Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 9.92

Table Chi-square value = 11.34 (alpha = 0.01)

Table Chi-square value = 7.81 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00

Used for Chi-square table value ==> df (#groups-1) = 3

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).

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survival (%)
File: 3104sn Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	1586.188	528.729	4.078
Within (Error)	12	1555.750	129.646	
Total	15	3141.938		

Critical F value = 3.49 (0:05,3,12)
Since F > Critical F REJECT Ho:All groups equal

survival (%)
File: 3104sn Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	93.500	93.500		
2	49	94.750	94.750	-0.155	
3	101	97.500	97.500	-0.497	
4	196	72.500	72.500	2.608	*

Dunnett table value = 2.29 (1 Tailed Value, P=0.05, df=12,3)

survival (%)
File: 3104sn Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	49	4	18.437	19.7	-1.250
3	101	4	18.437	19.7	-4.000
4	196	4	18.437	19.7	21.000

survival (%)
File: 3104sn Transform: NO TRANSFORMATION

Data Evaluation Report on the Toxicity of Ziram to Fathead Minnow (*Primephales promelas*), Early Life Cycle

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	93.500	93.500	95.250
2	49	4	94.750	94.750	95.250
3	101	4	97.500	97.500	95.250
4	196	4	72.500	72.500	72.500

survival (%)
 File: 3104sn 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	95.250				
49	95.250	0.217		1.78	k= 1, v=12
101	95.250	0.217		1.87	k= 2, v=12
196	72.500	2.608	*	1.90	k= 3, v=12

s = 11.386
 Note: df used for table values are approximate when v > 20.

hatching success (%)
 File: 3104h Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	97.5000	CALCULATED t VALUE =	-1.7321
GRP2 (BLANK CRTL) MEAN =	100.0000	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-2.5000		

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

hatching success (%)
 File: 3104h 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	1.608	5.808	9.168	5.808	1.608

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OBSERVED 0 9 6 9 0

Calculated Chi-Square goodness of fit test statistic = 7.8193
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

hatching success (%)
File: 3104h Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 587.500

W = 0.938

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

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

hatching success (%)
File: 3104h Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 18.67
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) = 3.00

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).

hatching success (%)
File: 3104h Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 9.14

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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) = 3.00
 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).

hatching success (%)
 File: 3104h Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	25262.500	5052.500	154.799
Within (Error)	18	587.500	32.639	
Total	23	25850.000		

Critical F value = 2.77 (0.05,5,18)
 Since F > Critical F REJECT Ho:All groups equal

hatching success (%)
 File: 3104h Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	97.500	97.500		
2	49	97.500	97.500	0.000	
3	101	98.750	98.750	-0.309	
4	196	95.000	95.000	0.619	
5	395	96.250	96.250	0.309	
6	750	10.000	10.000	21.660	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

hatching success (%)
 File: 3104h Transform: NO TRANSFORMATION

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

NUM OF Minimum Sig Diff % of DIFFERENCE

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GROUP	IDENTIFICATION	REPS	(IN ORIG. UNITS)	CONTROL	FROM CONTROL
1	neg control	4			
2	49	4	9.736	10.0	0.000
3	101	4	9.736	10.0	-1.250
4	196	4	9.736	10.0	2.500
5	395	4	9.736	10.0	1.250
6	750	4	9.736	10.0	87.500

hatching success (%)
File: 3104h 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	97.500	97.500	97.917
2	49	4	97.500	97.500	97.917
3	101	4	98.750	98.750	97.917
4	196	4	95.000	95.000	95.625
5	395	4	96.250	96.250	95.625
6	750	4	10.000	10.000	10.000

hatching success (%)
File: 3104h 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	97.917				
49	97.917	0.103		1.73	k= 1, v=18
101	97.917	0.103		1.82	k= 2, v=18
196	95.625	0.464		1.85	k= 3, v=18
395	95.625	0.464		1.86	k= 4, v=18
750	10.000	21.660	*	1.87	k= 5, v=18

s = 5.713

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	4.4E+02	2.5E+02	7.7E+02	0.12	0.56
EC10	4.7E+02	2.8E+02	7.7E+02	0.11	0.60
EC25	5.2E+02	3.6E+02	7.7E+02	0.081	0.68
EC50	5.9E+02	4.6E+02	7.6E+02	0.053	0.78

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Slope = 12.3 Std.Err. = 6.28

Goodness of fit: p = 0.97 based on DF= 3.0 22.

3104H : hatching success (%)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	98.8	97.8	1.00	100.	0.00
49.0	4.00	97.5	97.8	-0.250	100.	2.91e-14
101.	4.00	98.8	97.8	1.00	100.	2.91e-14
196.	4.00	95.0	97.8	-2.75	100.	1.72e-07
395.	4.00	96.3	96.2	4.36e-06	98.5	1.53
750.	4.00	10.0	10.0	1.37e-07	10.2	89.8

fish length

File: 31041

Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN = 21.4750 CALCULATED t VALUE = -1.2191
 GRP2 (BLANK CTRL) MEAN = 21.9250 DEGREES OF FREEDOM = 6
 DIFFERENCE IN MEANS = -0.4500

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

fish length

File: 31041

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	1.555	0.518	2.186
Within (Error)	12	2.845	0.237	
Total	15	4.400		

Critical F value = 3.49 (0.05,3,12)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

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fish length
File: 31041 Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	21.475	21.475		
2	49	21.800	21.800	-0.944	
3	101	20.950	20.950	1.525	
4	196	21.575	21.575	-0.290	

Dunnett table value = 2.29 (1 Tailed Value, P=0.05, df=12,3)

fish length
File: 31041 Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	49	4	0.788	3.7	-0.325
3	101	4	0.788	3.7	0.525
4	196	4	0.788	3.7	-0.100

fish length
File: 31041 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	21.475	21.475	21.408
2	49	4	21.800	21.800	21.408
3	101	4	20.950	20.950	21.408
4	196	4	21.575	21.575	21.575

fish length
File: 31041 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

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neg control	21.408				
49	21.408	0.194	1.78	k= 1, v=12	
101	21.408	0.194	1.87	k= 2, v=12	
196	21.575	0.290	1.90	k= 3, v=12	

s = 0.487

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

wet weight
File: 3104w Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	76.0000	CALCULATED t VALUE =	-1.5036
GRP2 (BLANK CRTL) MEAN =	82.1500	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-6.1500		

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
File: 3104w 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	1.072	3.872	6.112	3.872	1.072
OBSERVED	0	5	6	5	0

Calculated Chi-Square goodness of fit test statistic = 2.8033
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

wet weight
File: 3104w Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 286.135

W = 0.904

Critical W (P = 0.05) (n = 16) = 0.887

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Critical W (P = 0.01) (n = 16) = 0.844

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

wet weight
File: 3104w Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 9.73
Closest, conservative, Table H statistic = 120.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 4, df (# reps-1) = 3
Actual values ==> R (# groups) = 4, df (# avg reps-1) = 3.00

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
File: 3104w Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 3.12
Table Chi-square value = 11.34 (alpha = 0.01)
Table Chi-square value = 7.81 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 3

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
File: 3104w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	341.015	113.672	4.767

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Within (Error) 12 286.135 23.845

Total 15 627.150

Critical F value = 3.49 (0.05, 3, 12)
 Since F > Critical F REJECT Ho: All groups equal

wet weight
 File: 3104w Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	76.000	76.000		
2	49	83.225	83.225	-2.092	
3	101	72.800	72.800	0.927	
4	196	83.475	83.475	-2.165	

Dunnett table value = 2.29 (1 Tailed Value, P=0.05, df=12,3)

wet weight
 File: 3104w Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	49	4	7.907	10.4	-7.225
3	101	4	7.907	10.4	3.200
4	196	4	7.907	10.4	-7.475

wet weight
 File: 3104w 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	76.000	76.000	76.000
2	49	4	83.225	83.225	78.013
3	101	4	72.800	72.800	78.013
4	196	4	83.475	83.475	83.475

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wet weight
File: 3104w

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	76.000				
49	78.013	0.583		1.78	k= 1, v=12
101	78.013	0.583		1.87	k= 2, v=12
196	83.475	2.165	*	1.90	k= 3, v=12

s = 4.883

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

dry weight
File: 3104d

Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	13.7250	CALCULATED t VALUE =	-1.7989
GRP2 (BLANK CRTL) MEAN =	15.2000	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-1.4750		

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

dry weight
File: 3104d

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	1.340	4.840	7.640	4.840	1.340
OBSERVED	0	8	7	5	0

Calculated Chi-Square goodness of fit test statistic = 4.8020
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

dry weight

Data Evaluation Report on the Toxicity of Ziram to Fathead Minnow (*Primephales promelas*), Early Life Cycle

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Shapiro Wilks test for normality

D = 15.235

W = 0.950

Critical W (P = 0.05) (n = 20) = 0.905

Critical W (P = 0.01) (n = 20) = 0.868

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

dry weight

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Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 16.02

Closest, conservative, Table H statistic = 151.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) = 5,	df (# reps-1) = 3
Actual values ==>	R (# groups) = 5,	df (# avg reps-1) = 3.00

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).

dry weight

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Bartlett's test for homogeneity of variance

Calculated B statistic = 5.14

Table Chi-square value = 13.28 (alpha = 0.01)

Table Chi-square value = 9.49 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00

Used for Chi-square table value ==> df (#groups-1) = 4

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).

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dry weight
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	12.665	4.222	4.798
Within (Error)	12	10.555	0.880	
Total	15	23.220		

Critical F value = 3.49 (0.05,3,12)
Since F > Critical F REJECT Ho:All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	13.725	13.725		
2	49	15.075	15.075	-2.035	
3	101	12.650	12.650	1.621	
4	196	14.350	14.350	-0.942	

Dunnett table value = 2.29 (1 Tailed Value, P=0.05, df=12,3)

dry weight
File: 3104d Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	49	4	1.519	11.1	-1.350
3	101	4	1.519	11.1	1.075
4	196	4	1.519	11.1	-0.625

dry weight
File: 3104d Transform: NO TRANSFORMATION

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	13.725	13.725	13.725
2	49	4	15.075	15.075	13.863
3	101	4	12.650	12.650	13.863
4	196	4	14.350	14.350	14.350

dry weight
File: 3104d

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	13.725				
49	13.863	0.207		1.78	k= 1, v=12
101	13.863	0.207		1.87	k= 2, v=12
196	14.350	0.942		1.90	k= 3, v=12

s = 0.938

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

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APPENDIX 2: COPY OF REVIEWER'S TWA CALCULATIONS:

Nominal Concentration (ug ai/L)	Time (Day)	Measured Concentration (ug/L)	TWA (ug/L)
47	0	43.8	49
	7	49.9	
	14	49.0	
	21	48.6	
	28	50.6	
	33	47.2	
94	0	94.7	101
	7	104	
	14	106	
	21	101	
	28	96.5	
	33	101	
188	0	192	196
	7	198	
	14	192	
	21	199	
	28	206	
	33	180	
375	0	386	395
	7	415	
	14	383	
	21	396	
	28	386	
750	0	768	750
	7	731	