Data Evaluation Report on the Toxicity of Ziram to Sheepshead Minnow (*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 468564-01

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	D323418
OECD Data Point	{.....}
EPA MRID	468564-01
EPA Guideline	850.1400 (OPP §72-4a)

Test material: [^{14}C]Ziram **Radiochemical Purity:** 97%
Unlabeled Ziram Technical **Purity:** 98.2%
Common name Ziram
Chemical name: IUPAC: Zinc bis(dimethyldithiocarbamate)
CAS name: (*T*-4)-bis(Dimethylcarbamodithioato-κ*S*,κ*S*')zinc
CAS No.: 137-30-4
Synonyms: Ziram PHYTO

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature: Christie E. Padova
Date: 10/13/06

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.
Signature: Teri S Myers
Date: 10/23/06

Primary Reviewer: Allen Vaughan, Biologist
EPA/OPP/EFED/ERB – V
Date: 05/20/09

Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}
Date: {.....}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	034805	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Sutherland, C.A., T.Z. Kendall, and H.O. Krueger. 2006. Ziram: An Early Life-Stage Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegates*). Unpublished study performed by Wildlife International., Ltd., Easton, MD. Laboratory Project No. 602A-104. Study submitted by The Ziram Task Force, c/o Cerexagri, Inc., King of Prussia, PA. Study initiated February 17, 2006 and submitted June 2, 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.

EXECUTIVE SUMMARY:

The 34-day chronic toxicity of ziram to the early life stage of sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized eggs/embryos (80/level, ≤24 hours old) of sheepshead minnow were exposed to a mixture of radiolabelled plus unlabeled ziram at nominal concentrations of 0 (negative and solvent controls), 28, 56, 113, 225, and 450 µg ai/L. Mean measured concentrations were <LOQ, 27, 58, 117, 222 and 443 µg total residues/L. TWA concentrations were <8.29 (<LOQ, controls), 27, 58, 117, 224, and 445 µg total residues/L. The test system was maintained at 24.5-25.7 °C, pH of 7.9-8.0, and salinity of 20‰. As survival did not fall below 50% for any treatment level, the 34-day LC_{50} for post-hatch survival was >445 µg total residues/L. NOAEC and LOAEC values were 27 and 58 µg total residues/L, respectively, based on reduced size (length and dry weight) of newly-hatched larvae.

No treatment-related effects on time to hatch or hatching success were observed. Post-hatch survival and wet weight were statistically-reduced at the 443 µg total residues/L level compared to the negative control group. In addition, statistically-significant treatment-related effects on length and dry weight were observed at levels greater than and including the 58 µg total residues/L level. Regarding clinical observations, small fish were observed in the 117, 222, and 443 µg total residues/L treatment groups at the beginning of the post-hatch phase, but the majority appeared normal and healthy by Day 19 until test termination.

This study is scientifically sound and is classified as ACCEPTABLE.

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Embryos, ≤24 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

NOAEC: 27 µg total residues/L

LOAEC: 58 µg total residues/L

Endpoint(s) Affected: post-hatch clinical signs of toxicity, post-hatch survival, and growth
Most Sensitive Endpoint(s): growth (length and dry weight)

I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the 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 ^{14}C-Ziram

Description: Solids

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

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

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 20°C	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: Sheepshead minnow (*Cyprinodon variegates*) [*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: Collected by Aquatic Biosystems, Inc. and sent to the Wildlife International laboratory.

Source: Aquatic BioSystems, Inc., Fort Collins, CO

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. It was reported that the embryos were spawned from up to 30 adult males and 100 adult females. Upon arrival at the laboratory, the embryos were examined under a dissecting microscope to select healthy, viable specimens at approximately the same stage of development.
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)*

Parameter	Details	Remarks *Criteria*
Concentration of test material nominal: mean measured: TWA (reviewer-calculated):	0 (negative and solvent controls), 28, 56, 113, 225, and 450 µg ai/L <8.29 (<LOQ, controls), 27, 58, 117, 222, and 443 µg total residues/L, respectively <8.29 (<LOQ, controls), 27, 58, 117, 224, and 445 µg total residues/L, respectively	Total radioactive concentrations were determined using LSC at 0, 7, 14, 21, 28, and 34 days. All measured concentrations were within 20% among replicates. The radioactivity was not characterized to determine what percentage was parent material. *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)	Dimethyl formamide, 0.1 ml/L	*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/level	*Number of replicates should be 4 per concentration.* *A solvent control should be used in conjunction with a solubilizing agent.*

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 Continuous-flow serial diluter Approx. 10 volume additions per day N/A	The diluter was calibrated before and after the test and observed for normal operation twice daily during the test. *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 reatain 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	None reported.	*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	34 days (28-days post-hatch)	*Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.*

Parameter	Details	Remarks *Criteria*
Embryo cups, if used type/material (glass/stainless steel): size: fill volume:	 Glass cylinders with 425-µm nylon screen mesh attached to the bottom with silicone sealant Approx. 50 mm in diameter Not reported	The embryo cages were oscillated slowly to assure an adequate flow of media around the embryos. *Recommended embryo cups are 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 9 L 7 L (16.1-cm depth)	 *Recommended test vessel is all glass or glass with stainless steel frame.*
Source of dilution water	Natural sea water was collected at Indian River Inlet, DE and sand-filtered to remove particles >25 µm. The filtered water was diluted to a salinity of approximately 20‰ with fresh water from an on-site well, then aerated. Prior to delivery to the diluter system, the dilution water was filtered to 0.45 µm and UV-sterilized.	During the 4-week period immediately preceding the test, analysis of the dilution water yielded an average salinity of 19‰ and pH of 8.1. Results of periodic analysis for pesticides, organics, and metals were also provided (from water collected on 12/25/05); all pesticides and organics were below the LOD. The following metals were present: barium at 0.0074 mg/L, bromide at 38.9 mg/L, calcium at 228 mg/L, chloride at 12100 mg/L, magnesium at 779 mg/L, potassium at 269 mg/L, sodium at 5940 mg/L, and sulfate at 1500 mg/L. *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.*

Parameter	Details	Remarks *Criteria*
Water parameters 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 7.9-8.0 ≥5.9 mg/L (≥80% saturation) 24.5-25.7°C (all stages; maintained constant during the study) 16 hours light/8 hours dark, with 30-minute transition periods 20‰ N/A 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. Salinity was measured in one alternating replicate of the negative control and the highest concentration level at least weekly.	Light intensity at test initiation was 157 lux over one representative test chamber. *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 2 °C 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.*
Post-hatch details 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 test chamber:	Day 6, when hatching was at least 90% complete in the negative control chambers. All hatched larvae were released. Day 6	Survival ranged from 85-95% in the negative control replicates. *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.*

Parameter	Details	Remarks *Criteria*
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	 Day 6 Live brine shrimp nauplii (*Artemia sp.*) 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	Stable, as indicated by relatively constant measured concentrations (within 20% among replicate measurements).	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	86.8-110% of nominal Days 0, 7, 14, 21, 28, and 34 Not reported 8.29 µg total residues/L	Based on LSC analysis of test samples.
Positive control {if used, indicate the chemical and concentrations}	N/A	
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	- Embryo survival - Time to hatch - Larval survival - Measurement of growth (total length, wet weight, and dry weight) - Clinical signs of toxicity or abnormal behavior	*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	 Daily Daily Daily N/A Day 34 Not determined Daily	
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 6, hatching success averaged 91-100% in all test and control groups, with no treatment-related effect observed. The NOAEC for hatching success was 443 µg total residues/L.

On Day 34 (28 days post-hatch), fish survival averaged 93% in the negative control group, compared to 96, 93, 96, 92, 93, and 74% in the solvent control and mean-measured 27, 58, 117, 222, and 443 µg total residues/L groups, respectively. Fish survival was statistically-reduced ($p \leq 0.05$) at the 443 µg total residues/L level compared to the pooled control. The NOAEC for post-hatch survival was 222 µg total residues/L.

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

Treatment Mean Measured, µg total residues/L (and nominal, µg ai/L) concentrations	Egg hatched/embryo viability			Time to hatch, No.				Juvenile-survival on day 34	
	No. of eggs at study initiation	hatch/embryo viability		Day 4	Day 5	Day 6	Day 7	No. dead	% mortality
		No.	%						
Control (dilution water only)	80	73	91	5	44	73	73	5	7
Solvent control	80	74	93	7	45	74	74	3	4
27 (28)	80	74	93	7	49	74	74	5	7
58 (56)	80	73	91	7	54	73	73	3	4
117 (113)	80	76	95	12	44	73	76	6	8
222 (225)	80	80	100	17	56	80	80	6	8
443 (450)	80	76	95	8	59	76	76	20	26*
NOAEC		443 µg total residues/L		443 µg total residues/L				222 µg total residues/L	
EC_{50}		NR		NR				NR	
Positive control, if used mortality: EC_{50}: NOAEC	N/A	N/A		N/A				N/A	

NR – Not reported
* Statistically-significant difference from pooled control using Fisher's Exact test ($p \leq 0.05$).

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

Treatment Mean 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	17.7	68.0	16.1
Solvent control	N/A	N/A	N/A	17.6	69.4	15.9
27 (28)	N/A	N/A	N/A	17.5	69.3	15.6
58 (56)	N/A	N/A	N/A	16.9*	63.1*	14.0*
117 (113)	N/A	N/A	N/A	16.9*	63.3*	14.6*
222 (225)	N/A	N/A	N/A	17.1*	67.7	15.7
443 (450)	N/A	N/A	N/A	16.2**	57.1**	13.0**
NOAEC	N/A	N/A	N/A	222 µg total residues/L	222 µg total residues/L	222 µg total residues/L
LOAEC	N/A	N/A	N/A	443 µg total residues/L	443 µg total residues/L	443 µg total residues/L
EC_{50}	N/A	N/A	N/A	NR	NR	NR
Positive control, if used mortality: EC_{50}: NOAEC	N/A	N/A	N/A	N/A	N/A	N/A

[(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.
**Statistically-significant from pooled control using Bonferroni's test ($p \leq 0.05$).

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Daily observations of the sheepshead minnow embryos indicated that there were no apparent differences in time to hatch between the control groups and the ziram treatment groups. Embryos hatched on days 4, 5, and 6 and were released on day 6. An additional three embryos in the 117 µg total residues/L group hatched and were released on day 7. The NOAEC for time to hatch was 443 µg total residues/L.

The majority of fish in the control groups and in the ziram treatment groups appeared normal throughout the test. There were a few observations of organisms that appeared smaller in comparison to the majority of fish in the control replicates, and this effect was predominantly observed in newly-hatched larvae. The study authors reported that this effect occurred infrequently and was comparable in control and treatment groups (a NOAEC was not reported).

Statistically-significant reductions in total length were observed at the ≥58 µg total residues/L levels compared

to the pooled control. The study authors reported that differences in length that were <1 mm were not considered to be treatment related since this difference was within the error of measurement. Therefore, although statistically-different, differences from the 58, 117, and 222 µg total residues/L levels were not considered to be treatment-related. The difference at the 443 µg total residues/L level from the pooled control was 1.5 mm, and was considered relevant. The subsequent NOAEC for total length was reported to be 222 µg total residues/L. For wet and dry weights, statistically-significant reductions were observed from the pooled control at the 58, 117, and 443 µg total residues/L levels. The small differences in weights observed at the 58 and 117 µg total residues/L levels compared to the pooled control were not considered to be treatment related and did not follow an apparent concentration-dependent response. No statistically-significant differences were observed for wet or dry weights at the 222 µg total residues/L level. The only difference considered to be a treatment-related effect was the highest treatment group. Thus, the NOAEC for wet and dry weights was 222 µg total residues/L.

Table 5: Sub-lethal Effect of Ziram on Sheepshead Minnow[(a)].

Treatment Mean Measured, µg total residues/L (and nominal, µg ai/L) concentrations	Smaller in Size, Maximum % Occurrence[(a)]	Toxicity symptoms (specify)	Toxicity symptoms (specify)	Toxicity symptoms (specify)	Toxicity symptoms (specify)
Control (dilution water only)	0	N/A	N/A	N/A	N/A
Solvent control	0	N/A	N/A	N/A	N/A
27 (28)	4	N/A	N/A	N/A	N/A
58 (56)	1	N/A	N/A	N/A	N/A
117 (113)	4	N/A	N/A	N/A	N/A
222 (225)	12	N/A	N/A	N/A	N/A
443 (450)	16	N/A	N/A	N/A	N/A
NOAEC	Not Reported	N/A	N/A	N/A	N/A
LOAEC	Not Reported	N/A	N/A	N/A	N/A
Positive control, if used % sublethal effect: NOAEC:	N/A	N/A	N/A	N/A	N/A

[(a)] Reviewer-calculated as the maximum percentage of fish exhibiting effect during the entire post-hatch period.

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 was 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 the Shapiro-Wilks test, 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: Larval survival, length, wet and dry weight data were statistically analyzed. Results for hatching success could be visually determined because there were no reductions from the negative control group. 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 did not require transformation to satisfy the assumptions of ANOVA. The NOAEC values were determined using ANOVA, followed by Dunnett's or William's tests. These analyses were conducted using TOXSTAT statistical software. For all endpoints, the negative and solvent control groups were compared using a Student's t-test; no significant differences were detected and the negative control group was used for all comparisons to the treated groups.

NOAEC: 27 µg total residues/L
LOAEC: 58 µg total residues/L
Most sensitive endpoints: total length and dry weight

E. STUDY DEFICIENCIES:

This study is scientifically sound and provides useful data on the early life-stage toxicity of ziram to sheepshead 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 statistical conclusions differed from those of the study authors, but the results of the statistical analyses were identical. The study authors dismissed the significant effects on wet weight and length at the 58, 117, and 224 µg total residues/L levels because the overall responses were not dose-dependent and not considered to be biologically different from the pooled control groups. The reviewer compared all treatment responses to the negative control group. All growth parameters showed similar responses, with significantly reduced growth at the 58 and 117 µg total residues/L treatment levels, followed by a smaller magnitude reduction at the 224 µg total residues/L level and the greatest reduction at the highest treatment level (445 µg total residues/L). Given the consistency of these growth effects across length and weight parameters and the reported clinical observations noted up to Day 19 in the 117, 224, and 445 µg total residues/L levels, the reviewer chose not to dismiss the statistically significant adverse effects at levels up to and exceeding 58 µg total residues/L. As a result, the reviewer concluded that the NOAEC for this study is 27 µg total residues/L.

Biomass loading at the end of the test was 0.019 g fish/L/day (instantaneous 0.19 g fish/L), based on the negative control group.

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

In-life dates were February 23 – March 29, 2006.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. Based on treatment-related effects upon the size of newly-hatched larvae (length and dry weight) at levels equal to and exceeding 58 µg total residues/L, the NOAEC and LOAEC are 27 and 58 µg total residues/L, respectively.

LOAEC: 58 µg total residues/L

NOAEC: 27 µg total residues/L

Endpoint(s) Affected: post-hatch clinical signs of toxicity, post-hatch survival, and growth
Most Sensitive Endpoint(s): growth (length and dry weight)

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.1400: *Fish Early-Life Stage Toxicity Test.*

ASTM Standard E1241-98. 1998. *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish.* American Society for Testing and Materials.

U.S. Environmental Protection Agency. 1986. Standard Evaluation Procedure, *Fish Early Life-Stage Test.* Office of Pesticide Programs. Hazard Evaluation Division. EPA 540/9-86-138.

West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

The SAS System for Windows. 2001. Version 8.2. SAS Institute, Inc., Cary, North Carolina.

APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

survival
File: 6401s Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN	= 94.5000	CALCULATED t VALUE	= -0.7423
GRP2 (BLANK CRTL) MEAN	= 97.5000	DEGREES OF FREEDOM	= 6
DIFFERENCE IN MEANS	= -3.0000		

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: 6401s 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
OBSERVED	0	5	13	6	0

Calculated Chi-Square goodness of fit test statistic = 4.9364
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

survival
File: 6401s Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 779.500

W = 0.935

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.

survival

File: 6401s Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 4.29
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).

survival
File: 6401s Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 1.82
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).

survival
File: 6401s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1351.000	270.200	6.239
Within (Error)	18	779.500	43.306	
Total	23	2130.500		

Critical F value = 2.77 (0.05,5,18)

Since F > Critical F REJECT Ho:All groups equal

survival
File: 6401s 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	94.500	94.500		
2	27	92.750	92.750	0.376	
3	58	96.000	96.000	-0.322	
4	117	92.000	92.000	0.537	
5	224	92.500	92.500	0.430	
6	445	73.750	73.750	4.459	*

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

survival
File: 6401s 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	27	4	11.214	11.9	1.750
3	58	4	11.214	11.9	-1.500
4	117	4	11.214	11.9	2.500
5	224	4	11.214	11.9	2.000
6	445	4	11.214	11.9	20.750

survival
File: 6401s 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	94.500	94.500	94.500
2	27	4	92.750	92.750	94.375
3	58	4	96.000	96.000	94.375
4	117	4	92.000	92.000	92.250
5	224	4	92.500	92.500	92.250
6	445	4	73.750	73.750	73.750

survival
File: 6401s 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	94.500				
27	94.375	0.027		1.73	k= 1, v=18
58	94.375	0.027		1.82	k= 2, v=18
117	92.250	0.484		1.85	k= 3, v=18
224	92.250	0.484		1.86	k= 4, v=18
445	73.750	4.459	*	1.87	k= 5, v=18

s = 6.581
Note: df used for table values are approximate when v > 20.

length
File: 64011 Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	17.7250	CALCULATED t VALUE =	0.8704
GRP2 (BLANK CRTL) MEAN =	17.6000	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.1250		

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

length
File: 64011 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
OBSERVED	0	7	10	7	0

Calculated Chi-Square goodness of fit test statistic = 3.7808
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

```
length
File: 64011        Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =     1.297

W =     0.982

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.


length
File: 64011        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =    5.12
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).


length
File: 64011        Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =    2.47
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).

length
File: 64011 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	5.952	1.190	16.528
Within (Error)	18	1.297	0.072	
Total	23	7.250		

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

length
File: 64011 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	17.725	17.725		
2	27	17.450	17.450	1.449	
3	58	16.900	16.900	4.348	*
4	117	16.850	16.850	4.612	*
5	224	17.100	17.100	3.294	*
6	445	16.150	16.150	8.301	*

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

length
File: 64011 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	27	4	0.457	2.6	0.275
3	58	4	0.457	2.6	0.825
4	117	4	0.457	2.6	0.875
5	224	4	0.457	2.6	0.625
6	445	4	0.457	2.6	1.575

length
File: 64011 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	17.725	17.725	17.725
2	27	4	17.450	17.450	17.450
3	58	4	16.900	16.900	16.950
4	117	4	16.850	16.850	16.950
5	224	4	17.100	17.100	16.950
6	445	4	16.150	16.150	16.150

length
File: 64011 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	17.725				
27	17.450	1.449		1.73	k= 1, v=18
58	16.950	4.082	*	1.82	k= 2, v=18
117	16.950	4.082	*	1.85	k= 3, v=18
224	16.950	4.082	*	1.86	k= 4, v=18
445	16.150	8.296	*	1.87	k= 5, v=18

s = 0.268
Note: df used for table values are approximate when v > 20.

wet weight
File: 6401ww Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN = 68.0000 CALCULATED t VALUE = -0.8972
GRP2 (BLANK CRTL) MEAN = 69.4000 DEGREES OF FREEDOM = 6
DIFFERENCE IN MEANS = -1.4000

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: 6401ww 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
OBSERVED	0	7	9	8	0

Calculated Chi-Square goodness of fit test statistic = 4.2910
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

wet weight
File: 6401ww Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 186.442

W = 0.969

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.

wet weight
File: 6401ww Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 31.98
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).

wet weight
File: 6401ww Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 7.66
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).

wet weight
File: 6401ww Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	375.557	75.111	7.251
Within (Error)	18	186.442	10.358	
Total	23	562.000		

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

wet weight
File: 6401ww 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	68.000	68.000		
2	27	69.275	69.275	-0.560	
3	58	63.075	63.075	2.164	
4	117	63.250	63.250	2.087	
5	224	67.725	67.725	0.121	
6	445	57.750	57.750	4.504	*

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

wet weight
File: 6401ww 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	27	4	5.485	8.1	-1.275
3	58	4	5.485	8.1	4.925
4	117	4	5.485	8.1	4.750
5	224	4	5.485	8.1	0.275
6	445	4	5.485	8.1	10.250

wet weight
File: 6401ww 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	68.000	68.000	68.638
2	27	4	69.275	69.275	68.638
3	58	4	63.075	63.075	64.683
4	117	4	63.250	63.250	64.683
5	224	4	67.725	67.725	64.683
6	445	4	57.750	57.750	57.750

wet weight
File: 6401ww 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	68.638				
27	68.638	0.280		1.73	k= 1, v=18
58	64.683	1.457		1.82	k= 2, v=18
117	64.683	1.457		1.85	k= 3, v=18
224	64.683	1.457		1.86	k= 4, v=18
445	57.750	4.504	*	1.87	k= 5, v=18

s = 3.218
Note: df used for table values are approximate when v > 20.

dry weight
File: 6401dw Transform: NO TRANSFORM

```
       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
-------------------------------------------------------------------------------
-
 GRP1 (SOLVENT CRTL) MEAN =    16.0750     CALCULATED t VALUE =     0.5846
 GRP2 (BLANK CRTL) MEAN   =    15.8500     DEGREES OF FREEDOM =     6
 DIFFERENCE IN MEANS      =     0.2250
-------------------------------------------------------------------------------
-
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: 6401dw       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
OBSERVED     0             6                 10              8           0

-------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   4.1251
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


dry weight
File: 6401dw       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
-------------------------------------------------------------------------------

D =    7.950

W =    0.956

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.


dry weight
File: 6401dw       Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
-------------------------------------------------------------------------------
```

```
Calculated H statistic (max Var/min Var) =    3.98
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).

dry weight
File: 6401dw Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

```
Calculated B statistic =    2.27
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).

dry weight
File: 6401dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	27.823	5.565	12.590
Within (Error)	18	7.950	0.442	
Total	23	35.773		

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

dry weight

File: 6401dw 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	16.075	16.075		
2	27	15.600	15.600	1.010	
3	58	14.000	14.000	4.414	*
4	117	14.575	14.575	3.191	*
5	224	15.725	15.725	0.745	
6	445	13.025	13.025	6.488	*

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

dry weight
File: 6401dw 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	27	4	1.133	7.0	0.475
3	58	4	1.133	7.0	2.075
4	117	4	1.133	7.0	1.500
5	224	4	1.133	7.0	0.350
6	445	4	1.133	7.0	3.050

dry weight
File: 6401dw 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	16.075	16.075	16.075
2	27	4	15.600	15.600	15.600
3	58	4	14.000	14.000	14.767
4	117	4	14.575	14.575	14.767
5	224	4	15.725	15.725	14.767
6	445	4	13.025	13.025	13.025

dry weight
File: 6401dw 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	16.075				
27	15.600	1.011		1.73	k= 1, v=18
58	14.767	2.784	*	1.82	k= 2, v=18
117	14.767	2.784	*	1.85	k= 3, v=18
224	14.767	2.784	*	1.86	k= 4, v=18
445	13.025	6.490	*	1.87	k= 5, v=18

s = 0.665
Note: df used for table values are approximate when v > 20.

APPENDIX 2: COPY OF REVIEWER'S TWA CALCULATIONS:

Nominal Concentration (ug ai/L)	Time (Day)	Measured Concentration (ug/L)	TWA (ug/L)
28	0	30.0	
	7	29.0	
	14	28.1	
	21	24.8	
	28	25.9	
	34	24.3	
			27.04559
56	0	61.8	
	7	57.8	
	14	56.6	
	21	58.3	
	28	58.1	
	34	55.8	
			57.94853
113	0	124	
	7	118	
	14	115	
	21	119	
	28	116	
	34	108	
			116.94118
225	0	215	
	7	231	
	14	229	
	21	223	
	28	224	
	34	210	
			224.10294
450	0	441	
	7	453	
	14	460	
	21	443	
	28	432	
	34	430	
			445.10294