Data Evaluation Report on the Toxicity of AE 0317309 Technical (Pyrasulfotole) to Fathead Minnow (*Pimephales promelas*), Early Life Cycle

PMRA Submission Number 2006-2445 EPA MRID Number 468017-28

Data Requirement:	PMRA Data Code	9.5.3.1
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.2.4
	EPA MRID	468017-28
	EPA Guideline	850.1400

Test material: AE 0317309 Technical **Purity:** 95.4% ai
Common name Pyrasulfotole
Chemical name: IUPAC: (5-Hydroxy-1,3-dimethylpyrazol-4-yl)(α,α,α-trifluoro-2-mesyl-*p*-tolyl)methanone
CAS name: (5-Hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
CAS No.: 365400-11-9
Synonyms: None reported

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

Company Code	BCZ
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CITATION: Kern, M.E., and C.V. Lam. 2004. Early Life Stage Toxicity of AE 0317309 Technical to the Fathead Minnow (*Primephales promelas*) Under Flow-Through Conditions. Unpublished study performed by Bayer CropScience, Stilwell, KS. Laboratory Study No. EBAIX015 (A9841201). Study submitted by Bayer CropScience, Research Triangle Park, NC. Study initiated December 19, 2003 and submitted October 28, 2004.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies

that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 35-day chronic toxicity of AE 0317309 Technical (pyrasulfotole) to the early life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs/embryos (140/level, <24 hours old) of fathead minnow were exposed to nominal concentrations of 0 (negative control), 0.63, 1.25, 2.50, 5.00, and 10.0 mg ai/L. Mean-measured concentrations were <0.06 (<LOQ, control), 0.58, 1.10, 2.37, 5.07, and 10.6 mg ai/L. The test system was maintained at 24.6-25.8 °C and a pH of 7.4-8.2. The 35-day NOAEC, based on length (the most sensitive endpoint) was 0.58 mg ai/L.

No treatment-related effects on time to hatch, hatching success, or percent survival of alevins (assessed on day 5) were observed. Fry survival (assessed on day 35) was statistically-reduced at the 5.07 and 10.6 mg ai/L levels (66.3 and 13.8%, respectively) compared to the control (95.0%). In addition, morphological and behavioral effects were observed at these levels; effects included pale coloration, darkened coloration, bent spine, swimming erratically, fish at the water surface, loss of equilibrium, and fish resting on the bottom of the vessel.

A diluter malfunction occurred on day-22 (between 7am-4pm). The syringe delivering toxicant for test chamber stalled. Measurements of toxicant concentration were reduced for the samples this day and the following day; however, the system recovered and had no negative impact on the study. The analytical results remained consistent despite the diluter malfunction and therefore, the study is still acceptable.

Total length was the most sensitive endpoint, and was statistically-reduced compared to the control at all but the lowest level (0.58 mg ai/L). Total length averaged 23.9 mm for the control level, 23.4 mm for the 0.58 mg ai/L level, and 22.7, 22.0, 17.5, and 11.2 mm for the 1.10, 2.37, 5.07, and 10.6 mg ai/L levels, respectively. Mean dry weight was statistically-reduced at the 5.07 and 10.6 mg ai/L levels (27.4 and 4.0 mg, respectively) compared to the control (63.3 mg).

This study is scientifically sound, is classified as **ACCEPTABLE**, and satisfies guideline requirements for an early life stage toxicity study with fish.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Newly-fertilized embryos, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

NOAEC: 0.58 mg ai/L
LOAEC: 1.10 mg ai/L

Endpoint(s) affected: Fry survival, clinical effects, length, and dry weight
Most sensitive endpoint: Length

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series 72-4(a); the U.S. EPA Ecological Effects Test Guidelines, No. 850.1400; and OECD Guideline No. 210. No notable deviations from this guideline were observed.

1. A diluter malfunction occurred on day 21; corrective action was taken and did not impact the acceptability of the study.
2. Time-to-hatch: Average time to hatch for this species is five days; however, mean percent hatch on day 3 for concentration 5.07mg a.i./L was 15% (values for higher and lower concentrations were 0-2.2%). All calculations were based on day 5 percent hatch values (range 89.3-96.4%).
3. The pH (7.4-8.2) was slightly higher than recommended (7.2-7.6).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material	AE 0317309 Technical
Description:	Light brown powder
Lot No./Batch No. :	Op. 1-4
Purity:	94.5%
Stability of compound under test conditions:	Verified. Test solutions (all levels) were measured for AE 0317309 Technical concentrations on days 0, 7, 14, 21, 22, 28, and 35. Minimal variability was observed, with measured concentrations within 20% of mean values at all intervals.
Storage conditions of test chemicals:	Ambient conditions

Physicochemical properties of AE 0317309.

Parameter	Value	Comment
Molecular weight	362.3 g/mol	
Water Solubility (g/L) at 20°C	4.2 at pH 4 69.1 at pH 7 49.0 at pH 9	Very soluble
Vapor Pressure/Volatility	2.7×10^{-7} Pa at 20°C 6.8×10^{-7} Pa at 25°C	Non-volatile
UV Absorption	water $\lambda_{max} = 264$ 0.1M HCl $\lambda_{max} = 241$ 0.1M NaOH $\lambda_{max} = 216$	Not likely to undergo photolysis.
Pka	4.2 ± 0.15	
log K_{ow} at 23°C	0.276 at pH 4 -1.362 at pH 7 -1.58 at pH 9	Not likely to bioaccumulate
Stability of compound at room temperature, if provided		No significant degradation over 12 months at ambient temperatures.

Data obtained from pyrasulfatole chemistry review of Submission 2006-2445.

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

2. Test organism:

Species: Fathead minnow (*Pimephales promelas*)
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: <24 hours old
EPA recommends fish embryos 2 to 24 hours old.

Method of collection of the fertilized eggs: Embryos less than 24 hours old were removed from the spawning substrates and examined under a stereomicroscope to select healthy, viable specimens between the 2-cell stage and gastrulation.

Source: Laboratory cultures

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: None reported.

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)	 Continuous (for approx. 5 months) Same as test Not reported Healthy with no diseases observed.	Adult minnow were received from Osage Catfisheries, Inc. (Osage Beach, MO) on September 9, 2003. The breeding sub-culture consisted of aquarium containing 2 male and 5 female minnows, and three spawning substrates. Eggs were collected for the test on the morning of test initiation.
Number of fertilized eggs/embryos in each treatment at test initiation	140 embryos/treatment, divided into 35 embryos/cup, one cup/replicate aquarium, and four replicate aquaria/treatment	*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: measured:	 0 (negative control), 0.63, 1.25, 2.50, 5.00, and 10.0 mg ai/L <0.06 (<LOQ, control), 0.58, 1.10, 2.37, 5.07, and 10.6 mg ai/L	Measured concentrations were determined on days 0, 7, 14, 21, 22 (before and after discovering a diluter malfunction), 28, and 35. *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)	N/A	*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 N/A 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 Intermittent-flow proportional diluter (modified Mount-Brungs) Approximately 7 volume additions every 24 hours N/A	The flow-splitting accuracy (±10%) was verified prior to test initiation. The diluter system and syringe pump function were visually checked twice daily during the week and at least once daily on weekends. *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	No additional aeration was supplied.	*Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	35 days: 5-day hatching period and 30-day post-hatch period	OPPTS requires 32-day study (or 28-day post-hatch period) – OPPTS criteria met. *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:	 Not reported Not reported Not reported	The embryo cups were suspended in the water column and gently oscillated using a rocker arm. *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 8.4 L 7 L (25.2-cm depth)	 *Recommended test vessel is all glass or glass with stainless steel frame.*
Source of dilution water	Soft (40-60 mg/L as $CaCO_3$) dilution water was made by blending spring water with reverse-osmosis water. The spring water was filtered and UV-sterilized prior to blending with city water that had been dechlorinated, filtered, demineralized, and purified (reverse-osmosis). The blended water was intensely aerated and passed through a UV-sterilizer prior to use.	Results of the spring water and reverse-osmosis water for analysis of metals, inorganics, pesticides, and PCBs were provided (water analyzed on February 18, 2004). In addition, weekly monitoring of the dilution water for total suspended solids, unionized ammonia, and residual chlorine were provided. *Source of dilution water should be natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

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:	 48-62 mg/L as $CaCO_3$ 7.4-8.2 5.8-8.2 mg/L (70-99% saturation at 25°C) 24.6-25.8°C (all life stages) 16-hour light/8-hour dark cycle, with 30-minute transition periods N/A Alkalinity – 44-60 mg/L; conductivity – 131-168 µmhos Conductivity, alkalinity, hardness, and pH were measured at each level in one alternating replicate on days 0, 7, 14, 21, 28, and 35. DO was measured in at least one alternating replicate on days 0, 7, 14, 21, 22, 28, 30, and 35. Temperature was measured hourly in a centrally-located water bath, and also once daily.	Hardness and pH slightly exceeded recommendations. Light intensity averaged 68 foot candles (731 lux). *Recommended hardness: 40-48 mg/L as $CaCO_3$;* *Recommended pH:* 7.2 to 7.6 *Dissolved Oxygen (DO) should be measured at each concentration at least once a week;* *Freshwater parameters in a control and one concentration should be analyzed once a week.* *Temperature depends upon test species and should not deviate by more than 2EC from appropriate temperature.* *OECD recommends that DO concentration be between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*
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 80/level (20/replicate) Day 5	OPPTS specifies a control hatching success criterion of >66% and a post-hatch survival of 70%. Both criteria were achieved. *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 (*Artemia salina*) nauplii 0.5-5.0 ml per feeding At least once daily on weekends and three times daily on weekdays until approximately 24 hours prior to study termination.	
Stability of chemical in the test system	Stable, with measured concentrations at all treatment levels within 20% of mean-measured values.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	108 ± 6% 9 samples analyzed on a single day Not reported 0.06 mg ai/L	Based on method validation recoveries. During sample analysis, concurrently-analyzed laboratory spikes yielded recoveries of 99-108% of nominal concentrations.
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	The flow-through biomass loading factor was 0.1 g/L/24 hours.	Determined at the end of the study using control fish, whose mean wet weight was 0.28 g/fish.

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- Time to hatch - Hatchling success - Alevin/fry survival - Measurement of growth (length and dry weights) - Behavioral and morphological observations	*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 35 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:

The mean percent hatch [no. of alevin ÷ no. of eggs on day 0) x 100] was evaluated on day 5 and ranged from 89.3 to 96.4% in the control and all treatment groups, with no statistically-significant differences observed. Thus, the NOAEC was 10.6 mg ai/L.

Survivorship evaluated on day 5 [(no. of alevin + eggs on day 5 ÷ no. of eggs on day 0) x 100] averaged 94.3, 96.4, 90.7, 88.6, 89.3, and 92.8% for the control, 0.58, 1.10, 2.37, 5.07, and 10.6 mg ai/L levels, respectively, with no statistically-significant differences observed. On day 35 (study termination), fry survival averaged 95.0% in the control group, compared to 88.8-93.8% for the 0.58-2.37 mg ai/L levels, 66.3% for the 5.07 mg ai/L level, and 13.8% for the 10.6 mg ai/L level; differences were statistically-different from the control at the 5.07 and 10.6 mg ai/L levels. The NOAEC for fry survival was 2.37 mg ai/L. An EC_{50} value for fry survival was not determined.

Table 3: Effect of AE 0317309 Technical on egg hatching and survival at different life stage of fish.

Treatment (mg ai/L) Measured (and nominal) concentrations	Egg hatched/embryo viability			Time to hatch, % hatched			Juvenile-survival on day 35	
	No. of eggs at study initiation	hatch/embryo viability		day 3	day 4	day 5	No. dead[c]	% mortality
		No.[a]	%					
Control (dilution water only)	140	132	95.0	0.0	93.6	95.0	4	5.0
0.58 (0.63)	140	135	96.4	0.7	95.7	96.4	5	6.2
1.10 (1.25)	140	127	90.7	2.2	87.2	90.7	9	11.2
2.37 (2.5)	140	125	89.3	1.5	86.5	89.3	9	11.2
5.07 (5.0)	140	125	89.3	15.0[e]	87.1[e]	90.0	27	33.7*
10.6 (10.0)	140	130	94.3[b]	2.2	90.7	94.3	69	86.2[d]*
NOAEC	10.6 mg ai/L			10.6 mg ai/L			2.37 mg ai/L	
EC_{50}	>10.6 mg ai/L			>10.6 mg ai/L			Not determined	
Positive control, if used mortality: EC_{50}: NOAEC	N/A							

* Statistically-significant effect (p = 0.05).
[a] Calculated from raw data tables.
[b] For Replicate C, days 4 and 5 were incorrectly reported in the summary table (as 88.6%) causing incorrect mean reported values (of 89.3 and 92.1%, respectively). Actual % hatch for Replicate C on days 4 and 5 (determined from raw data tables) was 94.3 and 97.1%, respectively, resulting in mean % hatch values of 90.7 and 94.3%, respectively.
[c] Alevins were thinned to 80/level on day 5.
[d] Percent fry survival was incorrectly reported (as 12.5%) in the summary table; actual fry survival was 13.8%.
[e] On day 3, replicates A-C were incorrectly reported in the summary table (as 0.0, 0.0, and 57.1%) causing incorrect mean reported value (14.3%). Actual % hatch on day 3 for replicates A-C (determined from raw data tables) were 57.1, 2.9 and 0.0%, respectively, resulting in mean % hatch value of 15.0%. Additionally, for replicate C, day 4 was incorrectly reported (as 85.7%) causing incorrect mean reported value of 85.0%. Actual % hatch for replicate C on day 4 was 94.3%, resulting in mean % hatch value of 87.1%

Table 4: Effect of AE 0317309 Technical on Growth of Juvenile Fish

Treatment (mg ai/L) Measured (and nominal) concentrations	Swim-up			Growth -length (mm)	Growth-dry weight (mg)
	day x1	day x2	day xn		
Control (dilution water only)	N/A			23.9	63.3
0.58 (0.63)	N/A			23.4	65.3
1.10 (1.25)	N/A			22.7*	64.1
2.37 (2.5)	N/A			22.0*	57.5
5.07 (5.0)	N/A			17.5*	27.4*
10.6 (10.0)	N/A			11.2*	4.0*
NOAEC	N/A			0.58 mg ai/L	2.37 mg ai/L
LOAEC	N/A			1.10 mg ai/L	5.07 mg ai/L
EC_{50}	N/A			Not determined	Not determined
Positive control, if used mortality: EC_{50}: NOAEC	N/A				

N/A=not assessed
*Statistically different from controls (ANOVA w/ Dunnett's test; $p < 0.05$).

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Treatment-related clinical signs of toxicity were observed at the 5.07 and 10.6 mg ai/L levels. At the 5.07 mg ai/L, approximately 19 fish were noted to display effects including pale coloration, darkened coloration, bent spine, swimming erratically, fish at the water surface, and fish resting on the bottom of the vessel. At the 10.6 mg ai/L level, observations included pale coloration, loss of equilibrium, and fish on the bottom of the vessel (incidence not quantified due to high mortality). The NOAEC for clinical signs of toxicity was 2.37 mg ai/L.

Time to hatch began on day 3 (0-15.0% for all levels) and continued until day 5. By day 5, percent hatch ranged from 85.0 to 95.7% at all levels, with no treatment-related differences observed (determined visually). The NOAEC for time to hatch was 10.6 mg ai/L.

Total length was the most sensitive endpoint, and was statistically-reduced compared to the control at all levels except the lowest (of 0.58 mg ai/L). Total length averaged 23.9 mm for the control level, 23.4 mm for the 0.58 mg ai/L level, and 22.7, 22.0, 17.5, and 11.2 mm for the 1.10, 2.37, 5.07, and 10.6 mg ai/L levels, respectively. Mean dry weight was statistically-reduced at the highest two treatment levels, and averaged 63.3, 65.3, 64.1, 57.5, 27.4, and 4.0 mg for the control, 0.58, 1.10, 2.37, 5.07, and 10.6 mg ai/L levels, respectively. The NOAEC for growth, based on total length, was 0.58 mg ai/L.

C. REPORTED STATISTICS:

Survival (Days 5 and 35), hatching success (Day 5), total length (Day 35), and dry weights (Day 35) were statistically analyzed. Time to hatch was not statistically analyzed due to the relative consistency between the control and treatment levels, based on days 4 and 5 values. For all endpoints, replicate means were used for statistical analysis.

Data were fist assessed for normality using the Chi-square test and for homogeneity of variance using Bartlett=s test. All sets passed these assumptions, and the data were analyzed using ANOVA followed by Dunnett=s and William=s test (if appropriate).

The NOAEC and LOAEC were estimated based on effects data. All statistical analyses were conducted using mean-measured concentrations and the computer programs TOXSTAT ver. 3.3 or SAS ver. 8.0 or greater. Mean measured concentrations were used for all determinations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Survival (Days 5 and 35), hatching success (Day 5), total length (Day 35), and dry weights (Day 35) were statistically analyzed. All data satisfied the assumptions of normality and homogeneity of variances. The NOAEC and LOAEC values were determined using ANOVA, followed by William's test (when applicable) via Toxstat statistical software. Additionally, the EC_{50} values for survival at day 35, length, and dry weight (endpoints exhibiting at least 50% reduction from control) were determined using the Probit method via Nuthatch statistical software. Mean measured concentrations were used for all determinations.

Percent Hatch (day 5):
EC_{50}: >10.6 mg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 10.6 mg ai/L
LOAEC: >10.6 mg ai/L

Percent Survival (day 5):
EC_{50}: >10.6 mg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 10.6 mg ai/L
LOAEC: >10.6 mg ai/L

Percent Survival (day 35):
EC_{50}: 6.6 mg ai/L 95% C.I.: 6.2-7.1 mg ai/L
Probit Slope: 5.03 95% C.I.: 4.66-5.40
NOAEC: 2.37 mg ai/L
LOAEC: 5.07 mg ai/L

Length:
EC_{50}: 10.0 mg ai/L 95% C.I.: 9.3-11.0 mg ai/L
Probit Slope: 2.27 95% C.I.: 2.11-2.43
NOAEC: 0.58 mg ai/L
LOAEC: 1.10 mg ai/L

Dry Weight:
EC_{50}: 4.6 mg ai/L 95% C.I.: 4.2-5.0 mg ai/L
Probit Slope: 4.23 95% C.I.: 3.91-4.55
NOAEC: 2.37 mg ai/L
LOAEC: 5.07 mg ai/L

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWERS' COMMENTS:

The reviewers' conclusions were identical to the study authors'.

On day 22, a diluter malfunction occurred sometime between the morning diluter check (at 7:10 AM) and the afternoon diluter check (4:08 PM). It was determined that the syringe pump delivering toxicant had stalled. Test solutions from all levels were sampled immediately and then analyzed. Corrective action was quickly taken to insure that the diluter was operating correctly as soon as possible that afternoon (5:10 PM). Recoveries for samples taken prior to restarting the diluter were 82-103% of nominal concentrations. The samples taken that afternoon after restarting the diluter and allowing it to operate for a "period" were 87 -111% of nominal concentrations. Therefore, results indicated that the problem was resolved before it negatively impacted the study. Additionally, DO readings taken just after the malfunction was detected indicated DO levels >84% of saturation.

No undissolved test material was noted in the test system throughout the exposure period.

In-life dates for the definitive study were February 25 – March 31, 2004.

G. CONCLUSIONS:

This study is scientifically sound and is classified as **ACCEPTABLE**. AE 0317309 Technical adversely affected fry survival and growth of fathead minnow. Clinical effects were also observed at the two highest treatment levels. The most sensitive endpoint was total length, based on a NOAEC of 0.58 mg ai/L.

NOAEC: 0.58 mg ai/L
LOAEC: 1.10 mg ai/L

Endpoint(s) affected: Fry survival, clinical effects, length, and dry weight
Most sensitive endpoint: Length

III. REFERENCES:

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APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

percent hatch by day 5
File: 1728h' Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	165.180	33.036	1.338
Within (Error)	18	444.360	24.687	
Total	23	609.540		

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

percent hatch by day 5
File: 1728h' 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	control	94.975	94.975		
2	0.58	96.425	96.425	-0.413	
3	1.10	90.700	90.700	1.217	
4	2.37	89.275	89.275	1.622	
5	5.07	89.975	89.975	1.423	
6	10.6	92.150	92.150	0.804	

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

percent hatch by day 5
File: 1728h' 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	control	4			
2	0.58	4	8.467	8.9	-1.450
3	1.10	4	8.467	8.9	4.275
4	2.37	4	8.467	8.9	5.700
5	5.07	4	8.467	8.9	5.000
6	10.6	4	8.467	8.9	2.825

percent hatch by day 5
File: 1728h' Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	94.975	94.975	95.700
2	0.58	4	96.425	96.425	95.700
3	1.10	4	90.700	90.700	90.700
4	2.37	4	89.275	89.275	90.467
5	5.07	4	89.975	89.975	90.467
6	10.6	4	92.150	92.150	90.467

percent hatch by day 5
File: 1728h' 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
control	95.700				
0.58	95.700	0.206		1.73	k= 1, v=18
1.10	90.700	1.217		1.82	k= 2, v=18
2.37	90.467	1.283		1.85	k= 3, v=18
5.07	90.467	1.283		1.86	k= 4, v=18
10.6	90.467	1.283		1.87	k= 5, v=18

s = 4.969
Note: df used for table values are approximate when v > 20.

percent survivorship by day 5
File: 1728s5 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	193.368	38.674	1.448
Within (Error)	18	480.630	26.702	
Total	23	673.998		

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

percent survivorship by day 5
File: 1728s5 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	control	94.275	94.275		
2	0.58	96.425	96.425	-0.588	
3	1.10	90.700	90.700	0.978	
4	2.37	88.575	88.575	1.560	
5	5.07	89.275	89.275	1.368	
6	10.6	90.000	90.000	1.170	

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

percent survivorship by day 5
File: 1728s5 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	control	4			
2	0.58	4	8.806	9.3	-2.150
3	1.10	4	8.806	9.3	3.575
4	2.37	4	8.806	9.3	5.700
5	5.07	4	8.806	9.3	5.000
6	10.6	4	8.806	9.3	4.275

percent survivorship by day 5
File: 1728s5 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	94.275	94.275	95.350
2	0.58	4	96.425	96.425	95.350
3	1.10	4	90.700	90.700	90.700
4	2.37	4	88.575	88.575	89.283
5	5.07	4	89.275	89.275	89.283
6	10.6	4	90.000	90.000	89.283

percent survivorship by day 5
File: 1728s5 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
control	95.350				
0.58	95.350	0.294		1.73	k= 1, v=18
1.10	90.700	0.978		1.82	k= 2, v=18
2.37	89.283	1.366		1.85	k= 3, v=18
5.07	89.283	1.366		1.86	k= 4, v=18
10.6	89.283	1.366		1.87	k= 5, v=18

s = 5.167
Note: df used for table values are approximate when v > 20.

percent survivorship by day 35
File: 1728s35 Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	19821.875	3964.375	160.807
Within (Error)	18	443.750	24.653	
Total	23	20265.625		

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

percent survivorship by day 35
File: 1728s35 Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	95.000	95.000		
2	0.58	93.750	93.750	0.356	
3	1.10	88.750	88.750	1.780	
4	2.37	88.750	88.750	1.780	
5	5.07	66.250	66.250	8.189	*
6	10.6	13.750	13.750	23.142	*

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

percent survivorship by day 35
File: 1728s35 Transform: NO TRANSFORM

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	control	4			
2	0.58	4	8.461	8.9	1.250
3	1.10	4	8.461	8.9	6.250
4	2.37	4	8.461	8.9	6.250
5	5.07	4	8.461	8.9	28.750
6	10.6	4	8.461	8.9	81.250

percent survivorship by day 35
File: 1728s35 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	95.000	95.000	95.000
2	0.58	4	93.750	93.750	93.750
3	1.10	4	88.750	88.750	88.750
4	2.37	4	88.750	88.750	88.750
5	5.07	4	66.250	66.250	66.250
6	10.6	4	13.750	13.750	13.750

percent survivorship by day 35
File: 1728s35 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	95.000				
0.58	93.750	0.356		1.73	k= 1, v=18
1.10	88.750	1.780		1.82	k= 2, v=18
2.37	**88.750**	**1.780**		**1.85**	**k= 3, v=18**
5.07	66.250	8.189	*	1.86	k= 4, v=18
10.6	13.750	23.142	*	1.87	k= 5, v=18

s = 4.965
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	3.1	2.6	3.7	0.035	0.85
EC10	3.7	3.2	4.2	0.030	0.87
EC25	4.8	4.4	5.4	0.022	0.90
EC50	**6.6**	**6.2**	**7.1**	**0.015**	**0.93**

Slope = 5.03 Std.Err. = 0.368

Goodness of fit: p = 0.55 based on DF= 3.0 18.

1728S35 : percent survivorship by day 35

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	95.0	91.9	3.09	100.	0.00
0.580	4.00	93.8	91.9	1.84	100.	5.35e-06
1.10	4.00	88.8	91.9	-3.16	100.	0.00451
2.37	4.00	88.8	90.8	-2.01	98.7	1.26
5.07	4.00	66.2	66.0	0.289	71.8	28.2
10.6	4.00	13.8	13.8	-0.0447	15.0	85.0

mean length
File: 17281 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	485.643	97.129	206.219
Within (Error)	18	8.470	0.471	
Total	23	494.113		

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

mean length
File: 17281 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	control	23.925	23.925		

2	0.58	23.400	23.400	1.082	
3	1.10	22.725	22.725	2.473	*
4	2.37	22.000	22.000	3.967	*
5	5.07	17.525	17.525	13.188	*
6	10.6	11.225	11.225	26.170	*

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

mean length
File: 17281 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	control	4			
2	0.58	4	1.170	4.9	0.525
3	1.10	4	1.170	4.9	1.200
4	2.37	4	1.170	4.9	1.925
5	5.07	4	1.170	4.9	6.400
6	10.6	4	1.170	4.9	12.700

mean length
File: 17281 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	23.925	23.925	23.925
2	0.58	4	23.400	23.400	23.400
3	1.10	4	22.725	22.725	22.725
4	2.37	4	22.000	22.000	22.000
5	5.07	4	17.525	17.525	17.525
6	10.6	4	11.225	11.225	11.225

mean length
File: 17281 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
control	23.925				
0.58	**23.400**	**1.082**		**1.73**	**k= 1, v=18**
1.10	22.725	2.474	*	1.82	k= 2, v=18

2.37	22.000	3.969	*	1.85	k= 3, v=18
5.07	17.525	13.194	*	1.86	k= 4, v=18
10.6	11.225	26.183	*	1.87	k= 5, v=18

s = 0.686
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.9	1.5	2.4	0.053	0.78
EC10	2.7	2.2	3.3	0.042	0.82
EC25	5.0	4.5	5.6	0.024	0.89
EC50	**10.**	**9.3**	**11.**	**0.014**	**0.93**

Slope = 2.27 Std.Err. = 0.164

Goodness of fit: p = 0.50 based on DF= 3.0 18.

1728L : mean length

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	23.9	23.6	0.363	100.	0.00
0.580	4.00	23.4	23.5	-0.101	99.7	0.256
1.10	4.00	22.7	23.2	-0.483	98.5	1.50
2.37	4.00	22.0	21.7	0.292	92.1	7.87
5.07	4.00	17.5	17.6	-0.0801	74.7	25.3
10.6	4.00	11.2	11.2	0.00851	47.6	52.4

mean dry weight
File: 1728w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	12933.988	2586.798	126.204
Within (Error)	18	368.950	20.497	
Total	23	13302.938		

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

mean dry weight

File: 1728w 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	control	63.325	63.325		
2	0.58	65.375	65.375	-0.640	
3	1.10	64.050	64.050	-0.226	
4	2.37	57.525	57.525	1.812	
5	5.07	27.425	27.425	11.214	*
6	10.6	4.050	4.050	18.516	*

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

mean dry weight
File: 1728w 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	control	4			
2	0.58	4	7.715	12.2	-2.050
3	1.10	4	7.715	12.2	-0.725
4	2.37	4	7.715	12.2	5.800
5	5.07	4	7.715	12.2	35.900
6	10.6	4	7.715	12.2	59.275

mean dry weight
File: 1728w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	63.325	63.325	64.350
2	0.58	4	65.375	65.375	64.350
3	1.10	4	64.050	64.050	64.050
4	2.37	4	57.525	57.525	57.525
5	5.07	4	27.425	27.425	27.425
6	10.6	4	4.050	4.050	4.050

mean dry weight
File: 1728w 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
control	64.350				
0.58	64.350	0.320		1.73	k= 1, v=18
1.10	64.050	0.226		1.82	k= 2, v=18
2.37	**57.525**	**1.812**		**1.85**	**k= 3, v=18**
5.07	27.425	11.214	*	1.86	k= 4, v=18
10.6	4.050	18.516	*	1.87	k= 5, v=18

s = 4.527
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.9	1.5	2.3	0.045	0.81
EC10	2.3	1.9	2.8	0.039	0.83
EC25	3.2	2.8	3.7	0.029	0.87
EC50	**4.6**	**4.2**	**5.0**	**0.020**	**0.91**

Slope = 4.23 Std.Err. = 0.318

Goodness of fit: p = 0.97 based on DF= 3.0 18.

1728W : mean dry weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	63.3	64.4	-1.09	100.	0.00
0.580	4.00	65.4	64.4	0.965	100.	0.00721
1.10	4.00	64.0	64.1	-0.0861	99.6	0.433
2.37	4.00	57.5	57.2	0.329	88.8	11.2
5.07	4.00	27.4	27.6	-0.154	42.8	57.2
10.6	4.00	4.05	4.01	0.0362	6.23	93.8

percent hatch by day 5
6
4
4
4
4
4
4
control
94.3
91.4
97.1
97.1
0.58
97.1
88.6
100
100
1.10
91.4
88.6
91.4
91.4
2.37
97.1
85.7
85.7
88.6
5.07
80
97.1
97.1
85.7
10.6
94.3
91.4
88.6
94.3

percent hatch by day 5
6
4
4
4
4
4
4
control
94.3
91.4
97.1
97.1
0.58
97.1
88.6
100
100
1.10
91.4
88.6
91.4
91.4
2.37
97.1
85.7
85.7
88.6
5.07
80
97.1
97.1
85.7
10.6
94.3
91.4
88.6
94.3

percent survivorship by day 5
6
4
4
4
4
4
4
control
94.3000000
91.4000000
94.3000000
97.1000000
0.58
97.1000000
88.6000000
100.000000
100.000000
1.10
91.4000000
88.6000000
91.4000000
91.4000000
2.37
97.1000000
82.9000000
85.7000000
88.6000000
5.07
80.0000000
97.1
94.3000000
85.7000000
10.6
94.3000000
91.4000000
82.9000000
91.4000000

percent survivorship by day 5
6
4
4
4
4
4
4
control
94.3000000
91.4000000
94.3000000
97.1000000
0.58
97.1000000
88.6000000
100.000000
100.000000
1.10
91.4000000
88.6000000
91.4000000
91.4000000
2.37
97.1000000
82.9000000
85.7000000
88.6000000
5.07
80.0000000
97.1
94.3000000
85.7000000
10.6
94.3000000
91.4000000
82.9000000
91.4000000

mean dry weight
6
4
4
4
4
4
4
control
60.4
55.5
75.9
61.5
0.58
59.3
69.3
65.3
67.6
1.10
65.3
61
62.6
67.3
2.37
58.7
56.7
58.9
55.8
5.07
29.6
24
30.7
25.4
10.6
1.6
2.2
6.1
6.3