TEXT SEARCHABLE DOCUMENT - 2009

Data Evaluation Report on the Toxicity of BAS 800 H (Saflufenacil) to Fathead Minnow (*Pimephales promelas*), Early Life Cycle

PMRA Submission Number: 2008-0431
PMRA Document ID: 1547217 EPA MRID Number: 47127908

Data Requirement:	PMRA Data Code	9.5.3.1
	EPA DP Barcode	D349851
	OECD Data Point	IIA.8.2.4
	EPA MRID	47127908
	EPA Guideline	OPPTS 850.1400

Test material: BAS 800 H **Purity:** 93.8%
Common name Saflufenacil
Chemical name:
IUPAC: *N'*-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-pyrimidin-1-yl]benzoyl}-*N*-isopropyl-*N*-methylsulfamide
CAS: 2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2*H*)-pyrimidinyl]-4-fluoro-*N*-[[methyl(1-methylethyl)amino]sulfonyl]benzamide
CAS No.: 372137-35-4
Synonyms: None reported

Primary Reviewer: Christie E. Padova **Signature:** Christie E. Padova
Staff Scientist, Dynamac Corporation **Date:** 04/01/08

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental Inc. **Date:** 04/10/08

Primary Reviewer: Anita Pease **Date:** 06/09/09 APease 6/9/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Ann Lee **Date:** 06/09/09
HC-PMRA-EAD

Secondary Reviewer: Farzad Jahromi **Date:** 06/09/09
DEWHA-APVMA

Company Code	BAZ
Active Code	SFF
Use Site Category	13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code	118203

CITATION: Zok, S. 2007. BAS 800 H – Early Life-Stage Test on the Fathead Minnow (*Pimephales promelas*) in a Flow Through System (Including Amendment No. 1). Unpublished study performed by Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Report No. 51F0414/015150. Study sponsored by BASF Corporation, Research Triangle Park, NC. Study initiated February 2, 2006 and amendment submitted March 6, 2007.

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

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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 33-day chronic toxicity of BAS 800 H (Saflufenacil) to the early life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs/embryos (100/level, <6 hours old) of fathead minnow were exposed to a BAS 800 H at nominal concentrations of 0 (negative control), 0.1, 0.32, 1.0, 3.2, and 10 mg a.i./L (adjusted for purity). TWA concentrations were <0.001 (<LOQ, control), 0.100, 0.321, 0.997, 3.32, and 9.63 mg a.i./L, respectively. The test system was maintained at 25 ± 1°C and a pH of 7.8-8.0. The reviewer's analysis detected slight (5%), but statistically-significant reductions in embryo survival at the two highest treatment levels. No treatment-related effects were observed during the study on larval or juvenile survival, time to hatch or time to swim-up, or growth. In addition, no sub-lethal clinical signs of toxicity were observed. The 33-day LC_{50} was >9.63 mg a.i./L, and the NOAEC and LOAEC were 0.997 and 3.32 mg a.i./L, respectively, based on effects on embryo survival.

This study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for an early life toxicity study with fathead minnow.

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Embryos, <6 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

LOAEC: 3.32 mg a.i./L (TWA)
NOAEC: 0.997 mg a.i./L (TWA)

Endpoint(s) Affected: embryo survival at hatch (day 0)

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* (1992); U.S. EPA Series 850 – Ecological Effects Test Guidelines (draft), OPPTS No. 850.1400 *Fish Early Life-Stage Test* (1996); and U.S. EPA FIFRA 72-4(a) (1982) under consideration of the Standard Evaluation Procedure, *Fish Early Life-Stage Test* (1986). No deviations from OPPTS No. 850.1400 were observed.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was conducted in accordance with the GLP Principles of the OECD and of the German "Chemikaliengesetz" (Chemicals Act).

A. MATERIALS:

1. Test Material BAS 800 H

Description: Solid, light beige

Lot No./Batch No. : COD-000515

Purity: 93.8%

Stability of compound under test conditions: The stability of BAS 800 H was assessed at all treatment levels by sampling from all replicates on Day 0 and from alternating replicates weekly thereafter. All replicate recoveries were within 20% of mean-measured concentrations, indicating that concentrations of BAS 800 H were relatively maintained during the study.

Storage conditions of test chemicals: Room temperature

Physicochemical properties of saflufenacil.

Parameter	Values	Comments
Water solubility at 20°C	2.1 g/L	pH 7
Vapor pressure	4.5×10^{-15} Pa	20°C
UV absorption	272 nm	pH1/pH7
pKa	Neutral	Ambient pH
Kow	Log P_{ow} 2.6	20°C

(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: Embryos, <6 hours old, with confirmation
[*EPA recommends fish embryos 2 to 24 hours old.*]

Method of collection of the fertilized eggs: The eggs produced from 13 small groups of adult fathead minnow were carefully removed from the spawning tiles, washed, and pooled. Damaged eggs were removed, and a representative sample was examined using a stereomicroscope to ensure that they were in the blastula stage.

Source: Parental fathead minnow were supplied by Osage Catfisheries Inc., Osage Beach, MO.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: None reported. The concentrations used in the definitive study were selected in consultation with the sponsor.

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)	Not reported	

Parameter	Details	Remarks *Criteria*
Number of fertilized eggs/embryos in each treatment at test initiation	100 embryos/treatment level, divided into 25 embryos/vessel, 1 vessel/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)*
Concentration of test material Nominal: Mean measured: TWA (reviewer-calculated):	 0 (negative control), 0.1, 0.32, 1.0, 3.2, and 10 mg a.i./L <0.001 (<LOQ, control), 0.0972, 0.321, 0.980, 3.34, and 9.70 mg a.i./L, respectively <0.001 (<LOQ, control), 0.100, 0.321, 0.997, 3.32, and 9.63 mg a.i./L, respectively	Nominal concentrations were adjusted for the purity of the test substance. At all test levels, water samples were collected from each replicate chamber on Day 0, and from a single alternating replicate test chamber on Days 8, 15, 22, 29, and 33 TWA concentrations were reviewer-calculated using Excel software (worksheet copied in Appendix II). *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	

Parameter	Details	Remarks *Criteria*
		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.*
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. 5 volume additions per day N/A	The flow rates were calibrated before test initiation and confirmed during exposure. The apparatus was checked at least once daily for proper function. *Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.* *Toxicant Mixing:* *1) Mixing chamber is preferred;* *2) Aeration should not be used for mixing;* *3) The test solution should be completely mixed before introduction into the test system;* *4) Flow splitting accuracy should be within 10%.*
Aeration, if any	From Day 16 onward, slight	

Parameter	Details	Remarks *Criteria*
	aeration was administered to ensure sufficient oxygen content.	*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	33 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.*
Embryo cups, if used type/material (glass/stainless steel): size: fill volume:	 Cylindrical glass vessels, with plastic gauze-covered outlets Diameter of 19 cm with a 6-cm outlet height *Ca.* 1.7 L	The test solution was introduced into the vessel above the water surface. *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:	 Stainless steel aquaria, with stainless-steel gauze-covered outlets 29 x 21 x 22 cm, with a 15-cm overflow height *Ca.* 9 L	*Recommended test vessel is all glass or glass with stainless steel frame.*
Source of dilution water	The dilution water was non-chlorinated drinking water obtained from the municipal water works of the city of Frankenthal (hardness *ca.* 250 mg/L $CaCO_3$). The water was purified through a charcoal filter and diluted with de-ionized water to achieve a hardness of *ca.* 100 mg/L $CaCO_3$. The dilution water had a pH of	The drinking water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and BASF Aktiengesellschaft, as well as for the presence of microbes by a contract laboratory. Results from the September 20, 2005 analysis indicated that the drinking water was free of heavy metals and impurities and found suitable for toxicity testing.

Parameter	Details	Remarks *Criteria*
	7.8-7.9, a specific conductance of 254-268 µS, and a general total organic carbon content of <2.0 mg/L. On Day 7, the total organic carbon content measured 0.9 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.*
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:	98-104 mg/L as $CaCO_3$ 7.8-8.0 5.7-8.5 mg/L (68-101% saturation) 25 ± 1°C (maintained constant throughout study) Dim light (*ca.* 125-192 lux under the lid) at a cycle of 16 hours light/8 hours dark N/A Conductivity ranged from 254-268 µS and the acid capacity [K] ranged from 2.26-2.28 mmol/L Temperature was measured daily in alternating replicate aquaria and continuously in a control aquarium. DO and pH measurements were conducted twice weekly, the pH in alternating replicates and the DO in all replicates. Hardness was determined on Days 0 and 33 in all replicate vessels.	Water hardness and pH range requirements are not reported in OPPTS guidance. *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:	Day 5, when hatching was ≥95% complete in all treatment levels.	Control survival in the four replicates ranged from 92-100% (mean of 97%).

Parameter	Details	Remarks *Criteria*
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:	All hatched larvae were released. Day 15	*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.*
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	 Day 5 Newly-hatched brine shrimp larvae (artemia naupli) and from Day 7 onwards a fine-milled commercial diet (Tetramin, Tetrawerke, Melle, Germany) Increased in quantity with the duration of the study (otherwise not reported). At least twice daily. Feeding was continued until 1 day before study termination.	
Stability of chemical in the test system	Verified: analytical variation among all replicates for the same level were within 20% of mean-measured concentrations.	Fulfills OPPTS requirements.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	80.3-119.0% of nominal Days 0, 8, 15, 22, 29, and 33 Not reported 0.001 mg ai/L	Based on test sample recoveries.
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	

Parameter	Details	Remarks *Criteria*
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 at hatch - Larval survival from hatch to end of swim-up - Juvenile survival from end of swim-up to study termination - Time to hatch - Time to swim-up - Total length and wet 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 Daily Day 33 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:

No treatment-related effects on survival were observed during the study. For all treatment levels (including controls), embryo survival from test initiation through hatching ranged from 92-99%, larval survival from hatch until Day 6 ranged from 99-100%, juvenile survival from Days 6-33 ranged from 95-98%, and overall survival from Days 0-33 ranged from 88-96%. No statistically-significant differences were observed from the control performance at any level for these endpoints. The NOAEC for survival was reported by the study author to be 10 mg ai/L.

Table 3: Effect of BAS 800 H (Saflufenacil) on Egg Hatching and Survival at Different Life Stages of Fish.

Treatment TWA (and nominal) concentrations, mg a.i./L	Egg hatched/embryo viability			Start of hatch (Day)[a]	End of hatch (Day)	Juvenile-survival on day 33	
	No. of eggs at study initiation	hatch/embryo viability				No. surviving	% survival
		No.	%				
Control (dilution water only)	100	97	97	1	5	92	92
0.100 (0.1)	100	99	99	1	5	95	95
0.321 (0.32)	100	98	98	1	5	96	96
0.997 (1.0)	100	96	96	1	5	92	92
3.32 (3.2)	100	92	92	1	5	88	88
9.63 (10)	100	92	92	1	5	89	89
NOAEC		10.0 mg a.i./L		10.0 mg a.i./L		10.0 mg a.i./L	
EC_{50}		NR		NR		NR	
Positive control, if used mortality: EC_{50}: NOAEC		N/A		N/A		N/A	

[a] The start of hatch was defined as the day before the day on which the first hatched larva was observed, since hatch had taken place during the last day before the observation.
NR – Not reported

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Hatch in all replicates of the control and treatment levels began at Day 1 and was completed 4 days later (on Day 5). No treatment-related effect was observed. Similarly, the time to first swim-up was observed on Day 4 in the control and all treatment groups, and was completed at Day 6 at all levels. Swim-up occurred almost simultaneously in the control group and all concentration groups, with no treatment-related effect observed. The NOAEC for time to hatch and time to swim-up were reported to be 10.0 mg a.i./L.

No abnormal behavior, toxic signs, or morphological abnormalities were recorded during the study. The NOAEC for sub-lethal signs of toxicity was reported to be 10.0 mg a.i./L.

Growth was evaluated on day 33 in surviving fish by measuring the total length and wet weight. No statistically-significant differences were observed by the study author from the control performance for either growth endpoint. Mean wet weights ranged from 146-159 g for all control and treatment levels, and mean length averaged 2.4-2.5 cm for all control and treatment levels. The NOAEC for these endpoints was reported to be 10.0 mg a.i./L.

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

Treatment TWA (and nominal) concentrations, mg a.i./L	Start of swim-up (Days)	End of swim-up (Days)	Growth - length (cm)	Growth-wet weight (g)	Growth-dry weight (mg)
Control (dilution water only)	4	6	2.4	159	NR
0.100 (0.1)	4	6	2.4	151	NR
0.321 (0.32)	4	6	2.5	151	NR
0.997 (1.0)	4	6	2.4	150	NR
3.32 (3.2)	4	6	2.4	146	NR
9.63 (10)	4	6	2.4	149	NR
NOAEC	10.0 mg a.i./L	10.0 mg a.i./L	10.0 mg a.i./L	10.0 mg a.i./L	NR
LOAEC	>10.0 mg a.i./L	>10.0 mg a.i./L	>10.0 mg a.i./L	>10.0 mg a.i./L	NR
EC_{50}	NR	NR	NR	NR	NR
Positive control, if used % sublethal effect: NOAEC:	N/A	N/A	N/A	N/A	N/A

NR – not reported.

C. REPORTED STATISTICS:

Data that were statistically analyzed included hatching rate (Day 0-hatch), larval survival (hatch until end of swim-up on Day 6), juvenile survival (Days 6-33), overall survival (Days 0-33), mean total length of surviving fish at study termination, and the mean wet weight of surviving fish at study termination. The times to hatch and swim-up were visually evaluated.

For survival endpoints, the performance of the treatment groups was compared to the performance of the control group using a one-sided Fisher's exact test. For growth endpoint, comparisons were made using a two-sided Dunnett's test. In addition, all endpoints were compared using a one-sided Wilcoxon test to examine the variabilities between the replicates.

Results were provided in terms of nominal and mean-measured concentrations.

NOAEC: 10.0 mg a.i./L (9.70 mg a.i./L mean-measured)
LOAEC: >10.0 mg a.i./L (>9.70 mg a.i./L mean-measured)

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically verified the results for embryo hatch at day 0 and wet weight. The NOAEC and LOAEC for the remaining endpoints could be verified visually. Data were tested to determine if they satisfied the assumptions of normality and homogeneity of variances. The NOAEC and LOAEC values were determined using ANOVA, followed by William's test via Toxstat statistical software.

EC_{50}: >0.997 mg a.i./L (TWA) 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
LOAEC: 3.32 mg a.i./L (TWA)
NOAEC: 0.997 mg a.i./L (TWA)
Endpoint(s) Affected: embryo survival at hatch (day 0)

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results for embryo survival differed from the study author's. The reviewer's analysis detected significant reductions in this endpoint at the two highest treatment levels, while the study author's did not. The reviewer based the study NOAEC and LOAEC on these slight (5%), yet significant findings.

TWA concentrations were reviewer-calculated (refer to associated Excel worksheet in Appendix II). As TWA concentrations are more indicative of actual concentration levels, they were reported in the Executive Summary and Conclusions sections of the DER. TWA concentrations were calculated using the following equation:

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

where:

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

50 L of stock solution was prepared at 1062 mg test substance/L and the mixture was stirred over approximately 1 day using an ultra turrax device. Over the whole use period, the pH of the stock solution was checked daily and adjusted to 7 if necessary. Renewal of the stock solution in the stock solution tanks was done after 6/7 days.

Aqueous test samples were diluted with acetonitrile:water and acidified with formic acid prior to analysis by HPLC with MS detection. The limit of quantification was 0.001 mg/L BAS 800 H.

Low recoveries of BAS 800 H were obtained upon analysis of control samples collected on Days 0 (Replicates B and D), 8, and 15, which was considered to be caused by contamination of the sample rather than of the test solution. The two retained samples, which were drawn on the same time as the original sample, were sent to the analytical laboratory and it was confirmed that no test substance was present in the control group during the study.

The loading rates for all replicates of all test groups at the end of exposure ranged from 63.1-88.4 mg/L/day (*ca.* 0.7 g/L/day).

In-life dates were February 15 – March 20, 2006.

G. CONCLUSIONS:

This study is scientifically sound and is classified as ACCEPTABLE to the U.S. EPA and as FULLY RELIABLE to PMRA and APVMA. Significant reductions were detected for embryo survival at the two highest treatment levels. No other treatment-related effects were observed during the study. Based on embryo survival, the NOAEC and LOAEC for this study were 0.997 and 3.32 mg ai/L.

LOAEC: 3.32 mg a.i./L (TWA)
NOAEC: 0.997 mg a.i./L (TWA)

Endpoint(s) Affected: embryo survival at hatch (day 0)

III. REFERENCES:

A reference list was not provided.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
survival of hatched larvae (% of day 0)
File: 7908h        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              8                  9                7            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.


survival of hatched larvae (% of day 0)
File: 7908h        Transform: NO TRANSFORMATION

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

D =  232.000

W =    0.955

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 of hatched larvae (% of day 0)
File: 7908h        Transform: NO TRANSFORMATION

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

Calculated H statistic (max Var/min Var) =    8.00
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 of hatched larvae (% of day 0)
File: 7908h        Transform: NO TRANSFORMATION

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

Calculated B statistic =    3.70
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 of hatched larvae (% of day 0)
File: 7908h        Transform: NO TRANSFORMATION

                                ANOVA TABLE
-----------------------------------------------------------------------------

SOURCE             DF              SS                MS              F
-----------------------------------------------------------------------------
Between             5             181.333           36.267          2.814

Within (Error)     18             232.000           12.889
-----------------------------------------------------------------------------
Total              23             413.333
-----------------------------------------------------------------------------

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


survival of hatched larvae (% of day 0)
File: 7908h        Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2            Ho:Control<Treatment
-----------------------------------------------------------------------------
                                TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION           MEAN          ORIGINAL UNITS    T STAT  SIG
----- --------------------     -----------    ------------------  ------  ---
  1                 control      97.000             97.000
```

2	0.100	99.000	99.000	-0.788
3	0.321	98.000	98.000	-0.394
4	0.997	96.000	96.000	0.394
5	3.32	92.000	92.000	1.970
6	9.63	92.000	92.000	1.970

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

survival of hatched larvae (% of day 0)
File: 7908h 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	0.100	4	6.118	6.3	-2.000
3	0.321	4	6.118	6.3	-1.000
4	0.997	4	6.118	6.3	1.000
5	3.32	4	6.118	6.3	5.000
6	9.63	4	6.118	6.3	5.000

survival of hatched larvae (% of day 0)
File: 7908h Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	97.000	97.000	98.000
2	0.100	4	99.000	99.000	98.000
3	0.321	4	98.000	98.000	98.000
4	0.997	4	96.000	96.000	96.000
5	3.32	4	92.000	92.000	92.000
6	9.63	4	92.000	92.000	92.000

survival of hatched larvae (% of day 0)
File: 7908h 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	98.000				

```
              0.100      98.000      0.394                1.73        k= 1, v=18
              0.321      98.000      0.394                1.82        k= 2, v=18
              0.997      96.000      0.394                1.85        k= 3, v=18
               3.32      92.000      1.970       *        1.86        k= 4, v=18
               9.63      92.000      1.970       *        1.87        k= 5, v=18
-----------------------------------------------------------------------------
s =    3.590
Note: df used for table values are approximate when v > 20.

wet body weight (mg)
File: 7908w        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             10                  5               9           0

-------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   9.8908
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


wet body weight (mg)
File: 7908w        Transform: NO TRANSFORMATION

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

D = 2490.250

W =    0.959

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 body weight (mg)
File: 7908w         Transform: NO TRANSFORMATION

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

Calculated H statistic (max Var/min Var) =   12.43
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 body weight (mg)
File: 7908w        Transform: NO TRANSFORMATION

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

Calculated B statistic =     5.66
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 body weight (mg)
File: 7908w        Transform: NO TRANSFORMATION

                                ANOVA TABLE
--------------------------------------------------------------------------------

SOURCE              DF              SS                MS              F
--------------------------------------------------------------------------------
Between              5            348.708           69.742          0.504

Within (Error)      18           2490.250          138.347
--------------------------------------------------------------------------------
Total               23           2838.958
--------------------------------------------------------------------------------

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


wet body weight (mg)
File: 7908w        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	158.500	158.500		
2	0.100	151.250	151.250	0.872	
3	0.321	150.750	150.750	0.932	
4	0.997	150.000	150.000	1.022	
5	3.32	145.750	145.750	1.533	
6	9.63	149.500	149.500	1.082	

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

wet body weight (mg)
File: 7908w 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	0.100	4	20.044	12.6	7.250
3	0.321	4	20.044	12.6	7.750
4	0.997	4	20.044	12.6	8.500
5	3.32	4	20.044	12.6	12.750
6	9.63	4	20.044	12.6	9.000

wet body weight (mg)
File: 7908w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	158.500	158.500	158.500
2	0.100	4	151.250	151.250	151.250
3	0.321	4	150.750	150.750	150.750
4	0.997	4	150.000	150.000	150.000
5	3.32	4	145.750	145.750	147.625
6	9.63	4	149.500	149.500	147.625

wet body weight (mg)
File: 7908w 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	158.500				
0.100	151.250	0.872		1.73	k= 1, v=18
0.321	150.750	0.932		1.82	k= 2, v=18
0.997	150.000	1.022		1.85	k= 3, v=18
3.32	147.625	1.308		1.86	k= 4, v=18
9.63	147.625	1.308		1.87	k= 5, v=18

s = 11.762
Note: df used for table values are approximate when v > 20.

APPENDIX II: COPY OF REVIEWER'S TWA CALCULATIONS:

Time-Weighted Concentrations

Nominal Concentration (mg a.i./L)	Time (Day)	Measured Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	TWA (mg a.i./L)
0.1		0.0815		
		0.0831		
		0.0824		
	0	0.0816	0.08215	
	8		0.0958	
	15		0.0975	
	22		0.113	
	29		0.103	
	33		0.107	
			TWA	**0.1000333**
0.32		0.301		
		0.229		
		0.3		
	0	0.304	0.2835	
	8		0.329	
	15		0.332	
	22		0.319	
	29		0.324	
	33		0.336	
			TWA	**0.3215909**
1		0.879		
		0.883		
		0.879		
	0	0.875	0.879	
	8		1.01	
	15		0.913	
	22		1.09	
	29		1.03	
	33		1.06	
			TWA	**0.9968788**
3.2	0	3.23	3.235	
		3.26		
		3.22		

		3.23		
	8		3.3	
	15		3.07	
	22		3.46	
	29		3.48	
	33		3.59	
			TWA	**3.3248485**
10		9.6		
		9.58		
		9.59		
	0	9.69	9.615	
	8		8.92	
	15		8.03	
	22		11.9	
	29		9.61	
	33		10.1	
			TWA	**9.6340909**