

Data Evaluation Report on the Acute Toxicity of AMPA to Fish, *Oncorhynchus mykiss*
PMRA Submission Number {.....} EPA MRID Number 43334713

Data Requirement: PMRA Data Code {.....}
EPA DP Barcode Not Provided
OECD Data Point {.....}
EPA MRID 43334713
EPA Guideline OPPTS 850.1075 (72-1c)

Test material: Aminomethyl Phosphonic Acid (Glyphosate Degradate) **Purity:** 94.38%
Common name: AMPA
Chemical name: IUPAC: Not Reported
CAS name: Not Reported
CAS No. Not Reported
Synonyms: None Reported

Primary Reviewer: John Marton
Staff Scientist, Cambridge Environmental Inc.

Signature: 
Date: 1/11/07

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.

Signature: 
Date: 2/20/07

Primary Reviewer: Stephen Carey
EPA Biologist, OPP, EFED

Date: 7/17/07

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 417300 (Parent Compound) & 207800 (Degradate Compound)

Date Evaluation Completed: July 17 2007

CITATION: Bowman, J.H. 1991. Acute Toxicity of AMPA to Rainbow Trout (*Oncorhynchus mykiss*). Unpublished study performed by ABC Laboratories, Inc., Columbia, Missouri. Laboratory project ID 38987. Study sponsored by Monsanto Agricultural Company, St. Louis, Missouri. Study completed on June 18, 1991.

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

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EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, Rainbow trout (*Oncorhynchus mykiss*) were exposed to AMPA (Glyphosate Degradate) at nominal concentrations of 0 (negative control), 30.2, 52.9, 94, 170, 302, 529 and 944 mg ai/L under static conditions; mean-measured concentrations were 0 (negative control), 30.8, 53.5, 97, 174, 306, 539 and 991 mg ai/L. The mortality 96-h LC₅₀ and NOAEC were 499 mg a.i/L and 306 mg a.i./L, respectively. The LOAEC and NOAEC values, based on sub-lethal effects, were 306 and 174 mg ai/L, respectively. Sub-lethal effects (quiescence, surfacing, on bottom of the test vessel, labored respiration, erratic swimming, loss of equilibrium, dark discoloration, light discoloration and excitement) were observed in the groups exposed to 52.9-944 mg ai/L of AMPA. Based on the results of this study, AMPA (Glyphosate Degradate) would be classified as practically non-toxic to Rainbow trout (*Oncorhynchus mykiss*) in accordance with the classification system of the U.S. EPA.

This toxicity study is classified scientifically sound and satisfies the guideline requirement for an acute freshwater fish toxicity study with *Oncorhynchus mykiss*.

Results Synopsis

Test Organism Size/Age (mean weight or length): Wet Weight- 0.79 (\pm 0.19) g; Standard Length- 39 (\pm 3) mm;
Based on 10 control fish at test termination.

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

Mortality

LC₅₀: 499 mg ai/L 95% C.I.: 391-647 mg ai/L

NOAEC: 306 mg ai/L

Probit Slope: 6.42 95% C.I.: 2.95-9.89

EC₅₀: Not Determined

Sub-lethal Effects

LOAEC: 306 mg ai/L (visual observation)

NOAEC: 174 mg ai/L (visual observation)

Endpoint(s) Affected: Mortality and Sub-Lethal Effects

I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in U.S. EPA, Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, Ecological Research Series, EPA-660/3-75-009 and American Public Health Association, Standard Methods for the Examination of Water and Wastewater. The following deviations from OPPTS 850.1075 were noted:

1. The Lot/Batch number of the test material was not provided.
2. The physiochemical properties of the test material were not reported.
3. Pre-test mortality of the test organisms was not reported.
4. The pH decreased with increasing concentration to yield values of 4.2-7.6; recommended pH values are 7.2-7.6.
5. Water quality parameters were inadvertently missed at 48 hours and temperature was not continually monitored in the negative control vessel.

The deviations were considered minor and did not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. The exposure phase of the definitive toxicity test was conducted in compliance with EPA GLP Standards, 40 CFR 160. The analytical phase of the definitive test was not conducted under and current GLP standards.

A. MATERIALS:

1. Test material Aminomethyl Phosphonic Acid (Glyphosate Degradate)

Description: White Powder

Lot No./Batch No. : Not Reported

Purity: 94.38%

Stability of compound under test conditions: Analytical verification of the test material in the dilution water was conducted at 0 and 96 hours and yielded recoveries of 101-105% of nominal. Concurrently analyzed QC samples yielded recoveries of 97.3-101% of nominal.

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

Storage conditions of test chemicals: Stored at room temperature.

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Physicochemical properties of AMPA.

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

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)
Age at test initiation: Juvenile
Weight at study initiation: 0.79 (\pm 0.19) g; Based on control fish at test termination
Length at study initiation: 39 (\pm 3) mm; Based on control fish at test termination
Source: Mt. Lassen Trout Farms, Red Bluff,

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Two 96 hour static range-finding tests were conducted prior to the definitive test. The first test exposed fish to nominal concentrations of 1, 10 and 100 mg/L (five fish per concentration). No abnormal effects were observed at 1 and 10 mg/L and one fish died at the 100 mg/L treatment level. The second test was conducted as a limit test with a nominal concentration of 180 mg/L to determine if the 100 mg/L concentration was near the LC₅₀; mortality was 0%. Based on the results from preliminary testing, 7 nominal concentrations of the test compound, ranging in a logarithmic series from 32 to 1000 mg/L (30.2-944 mg ai/L; reviewer-calculated), with ten fish per concentration, were selected for the definitive toxicity test.

b. Definitive Study

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Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
<u>Acclimation</u>		
Period:	Fish were received as eggs and were acclimated to approximate test temperatures for 6 weeks. A sub-lot of fish were acclimated to test temperatures for 72 hours prior to testing.	<p><i>The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.</i></p>
Conditions: (same as test or not)	Same as test for 72 hours.	
Feeding:	Fish food (Zeigler Bros.) or newly hatched brine shrimp were provided daily. Food was withheld for 72 hours prior to, and during, testing.	
Health: (any mortality observed)	Fish appeared normal and healthy; mortality was not reported.	
Duration of the test	96 hours	<p><i>The recommended test duration is 96 hours.</i></p>
<u>Test condition</u>		
Static/flow-through	Static	<p><i>A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.</i></p>
Type of dilution system - for flow-through method.	N/A	
Renewal rate for static renewal	N/A	
Aeration, if any	None provided	<p><i>Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.</i></p>

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Parameter	Details	Remarks
		<i>Criteria</i>
<p><u>Test vessel</u></p> <p>Material: (glass/stainless steel)</p> <p>Size:</p> <p>Fill volume:</p>	<p>Glass</p> <p>19 L</p> <p>15 L</p>	<p><i>Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.</i></p> <p><i>Fill volume is usually 15-30 L of solution.</i></p>
<p>Source of dilution water</p> <p>Quality:</p>	<p>Soft blended water was prepared by blending naturally hard well water with well water that had been demineralized by reverse osmosis.</p>	<p><i>Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water</i></p> <p><i>(http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.</i></p>

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Parameter	Details	Remarks ----- Criteria
<p><u>Water parameters:</u> Hardness</p> <p>pH</p> <p>Dissolved oxygen</p> <p>Total Organic carbon</p> <p>Particulate Matter</p> <p>Metals</p> <p>Pesticides</p> <p>Chlorine</p> <p>Temperature</p> <p>{Salinity for marine or estuarine species}</p> <p>Intervals of water quality measurement</p>	<p>40-48 mg/L as CaCO₃</p> <p>4.2-7.6</p> <p>7.1-9.4 mg/L (69-91% saturation)</p> <p><1.0 mg/L</p> <p>1.0 mg/L as total suspended solids</p> <p>See Reviewer's Comments</p> <p>None detected</p> <p>Not Reported</p> <p>12°C</p> <p>N/A</p> <p>Temperature, DO and pH were measured in all test vessels at 0, 24, 72 and 96 hours.</p>	<p>-----</p> <p><u>Hardness:</u> EPA recommends 40 - 48 mg/L as CaCO₃ (OECD recommends 10 - 250 mg/L)</p> <p><u>pH:</u> EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)</p> <p><u>Dissolved Oxygen:</u> EPA recommends: Static: > 60% during first 48 hrs and > 40% during second 48 hrs; flow-through: >60%; (OECD guideline recommends at least 80% saturation value).</p> <p><u>Temperature:</u> EPA recommends 12 °C for coldwater species, 17 or 22 °C for warmwater species, and 22 ± 1 °C for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).</p> <p><u>Salinity:</u> EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.</p> <p>Water quality should be measured at beginning of test and every 48 hours.</p>
<p><u>Number of replicates/groups:</u> control: solvent control: treated ones:</p>	<p>1 N/A 1</p>	<p>A solvent control was not used.</p> <p>-----</p> <p>Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.</p>
<p><u>Number of organisms per replicate /groups:</u> control: solvent control: treated ones:</p>	<p>10 N/A 10</p>	<p>-----</p> <p>Number of organisms per replicate should be ~ 10/concentration; OECD guideline recommends at least 7 fish/concentration.</p>

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Parameter	Details	Remarks
		Criteria
Biomass loading rate	0.53 g/L	<i>Recommended static conditions are #0.8 g/L at #17°C and #0.5 g/L at > 17°C. Recommended flow-through conditions are #1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.</i>
<u>Test concentrations:</u> nominal: measured:	0 (negative control), 30.2, 52.9, 94, 170, 302, 529 and 944 mg ai/L 0 (negative control), 30.8, 53.5, 97, 174, 306, 539 and 991 mg ai/L	Nominal and mean-measured concentrations were corrected for the purity of the test material (94.38%) by the reviewer.
Solvent (type, percentage, if used)	N/A; a solvent was not used.	<i>The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.</i>
Lighting	16L:8D with a 30 minute transitional period.	Light intensity averaged 110 footcandles. <i>The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.</i>
Feeding	No food was provided during the test.	<i>Fish should not feed during the study.</i>
<u>Recovery of chemical</u> Frequency of determination Level of quantization Level of detection	0 and 96 hours. Not Reported Not Reported	
Positive control {if used, indicate the chemical and concentrations }	N/A; a positive control was not used	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks
		<i>Criteria</i>
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	3, 24, 48, 72 and 96 hours	<i>Observation intervals should be a minimum of every 24 hours.</i>
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

By test termination, mortality was 0% in the negative control and mean-measured 30.8-306 mg ai/L treatment groups, and 80 and 90% in the mean-measured 539 and 991 mg ai/L treatment groups, respectively. The resulting LC₅₀ value was 520 (410-660) mg/L, based on the study author's analysis. Corrected for the purity of the test material, the study author's LC₅₀ (and 95% C.I.) value was 491 (387-623) mg ai/L.

Table 3: Effect of AMPA on Mortality of *Oncorhynchus mykiss*.

Treatment (mg a.i./L) Mean-Measured And (Nominal)	No. of Fish at Start of Study	Observation Period					
		Day 1		Day 3		Day 4	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
0 (Negative Control)	10	0	0	0	0	0	0
30.8 (30.2)	10	0	0	0	0	0	0
53.5 (52.9)	10	0	0	0	0	0	0
97 (94)	10	0	0	0	0	0	0
174 (170)	10	0	0	0	0	0	0
306 (302)	10	0	0	0	0	0	0
539 (529)	10	0	0	2	20	8	80
991 (944)	10	1	10	7	70	9	90
NOAEC	306 mg ai/L						
LC ₅₀	491 (387-623) mg ai/L*						
Positive control, if used mortality: LC ₅₀ :							

* Corrected for the purity of the test material (94.38%) by the reviewer.

B. NON-LETHAL TOXICITY ENDPOINTS:

With the exception of the negative control and the mean-measured 30.8 mg ai/L treatment group, labored respiration was observed in all treatment groups after 3 hours of exposure. One fish in the mean-measured 306 mg ai/L treatment group was observed to be excitable. In the 539 mg ai/L treatment group, fish were exhibiting surfacing, lying on the bottom of the test vessel and excitement, in addition to the labored respiration. At the highest treatment level, surfacing, excitement, labored respiration and lying on the bottom of the test vessel were observed. All fish in the mean-measured 53.5 and 97 mg ai/L treatment groups appeared normal and healthy from 24-96 hours. By test termination, effects were only observed in the mean-measured 306-991 mg ai/L treatment groups; observed effects included surfacing, loss of equilibrium, labored respiration, lying on the bottom of the test vessel, quiescence and dark discoloration. Based on the sub-lethal observations, the NOAEC value was 174 mg ai/L.

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Table 4: Sub-lethal Effect of AMPA on *Oncorhynchus mykiss*.

Treatment (mg a.i./L) Mean-Measured and (Nominal)	Observation Period		
	Endpoints at Day 1	Endpoints at Day 3	Endpoints at Day 4
	% Affected	% Affected	% Affected
0 (Negative Control)	A.N.	A.N.	A.N.
30.8 (30.2)	A.N.	A.N.	A.N.
53.5 (52.9)	A.N.	A.N.	A.N.
97 (94)	A.N.	A.N.	A.N.
174 (170)	10% - On bottom of test vessel and quiescent	10% - Labored respiration	A.N.
306 (302)	100% - Labored respiration	20% - Light discoloration 20% - On bottom of test vessel 30% - Surfacing, loss of equilibrium 30% - On bottom of test vessel, loss of equilibrium, labored respiration and excitable	10% - Surfacing, loss of equilibrium and labored respiration 90% - On bottom of test vessel, loss of equilibrium, labored respiration and quiescent
539 (529)	40% - On bottom of test vessel and quiescent 10% - Loss of equilibrium and erratic swimming 30% - Excitable	100% - On bottom of test vessel, loss of equilibrium and labored respiration	100% - Labored respiration, loss of equilibrium, on bottom of test vessel, quiescent and dark discoloration
991 (944)	67% - Light discoloration 33% - On bottom of test vessel, quiescent and light discoloration	33% - Surfacing, loss of equilibrium and erratic swimming 67% - Quiescent, on bottom of test vessel, labored respiration and loss of equilibrium	100% - On bottom of test vessel, loss of equilibrium, labored respiration, quiescent and dark discoloration
NOAEC	174 mg ai/L		
LOAEC	306 mg ai/L		
EC ₅₀	Not Reported		
Positive control, if used % sublethal effect: EC ₅₀ :	N/A	N/A	N/A

A.N.-All surviving fish appear normal and healthy

N/A- Not applicable

C. REPORTED STATISTICS:

The computerized LC₅₀ program developed by Stephan *et al.* was employed the probit method to analyzed the effect of concentration versus cumulative mortality. The study author's toxicity values were determined using the uncorrected nominal concentrations. The reviewer corrected these values for the purity of the test material for the purposes of comparing the results to those of the reviewer.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The mortality 96-hour LC₅₀ value was determined using the probit method via Toxanal Statistical software. The 96-hour NOAEC value was determined visually based on the observed mortality and sub-lethal effects. All toxicity values were based on the mean-measured concentrations corrected for the purity of the test material.

Mortality

LC₅₀: 499 mg ai/L 95% C.I.: 391-647 mg ai/L

NOAEC: 306 mg ai/L

Probit Slope: 6.42 95% C.I.: 2.95-9.89

EC₅₀: Not Determined

Sub-lethal Effects

LOAEC: 306 mg ai/L (visual observation)

NOAEC: 174 mg ai/L (visual observation)

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results calculated using the corrected mean-measured concentrations agreed with those of the study author and are reported in the Executive Summary and Conclusions sections of this DER.

A full description of the analytical method and results is provided in MRID 43334714. Holland, M.E. 1994. Results of the Analyses of AMPA in a 96-Hour Acute Study with Rainbow Trout. Guideline 72-1.

The results of the periodic screening analysis of the dilution water indicated the presence of the following elements: boron (0.314 mg/L), copper (0.04 mg/L), fluoride (0.19 mg/L), lead (5.3 µg/L) and zinc (0.101 mg/L).

The in-life portion of the definitive toxicity test was conducted from October 26 to October 30, 1990.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. The 96-hour LC₅₀ and NOAEC values were 499 and 174 mg ai/L, respectively.

Mortality

LC₅₀: 499 mg ai/L 95% C.I.: 391-647 mg ai/L

NOAEC: 306 mg ai/L

Probit Slope: 6.42 95% C.I.: 2.95-9.89

EC₅₀: Not Determined

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Sub-lethal Effects

LOAEC: 306 mg ai/L (visual observation)

NOAEC: 174 mg ai/L (visual observation)

Endpoint(s) Affected: Mortality and Sub-Lethal Effects

III. REFERENCES:

- Brauhn, J.L and R.A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975, 45 p.
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- American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th Ed. Washington, DC. 1134 p.
- Stephan, C.E. K.A. Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for calculating an LC₅₀- U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August 1978.
- Stephan, C.E. 1977. Methods for Calculating an LC₅₀, p. 65-84. In F.L. Mayer and J.L. Hamelink (eds). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.
- U.S. Environmental Protection Agency. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 54, No. 158:34067-34074.
- Organization for Economic Cooperation and Development, 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C (81) 30 (final): 7-28.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
991	10	9	90	1.074219
539	10	8	80	5.46575
306	10	0	0	9.765625E-02
174	10	0	0	9.765625E-02
97	10	0	0	9.765625E-02
53.5	10	0	0	9.765625E-02
30.8	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 306 AND 991 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 451.0268

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.173944	529.2694	404.1935	774.9557

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
21	.2923539	1	.4445892

SLOPE = 6.417251
 95 PERCENT CONFIDENCE LIMITS = 2.947459 AND 9.887043

LC50 = 499.4882
95 PERCENT CONFIDENCE LIMITS = 391.3569 AND 647.0542

LC10 = 316.6802
 95 PERCENT CONFIDENCE LIMITS = 173.9763 AND 401.5073