Data Evaluation Report on the Acute Toxicity of AMPA to Freshwater Invertebrates – *Daphnia magna*

PMRA Submission Number {.....} EPA MRID Number 43334715

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	Not Provided
	OECD Data Point	{.....}
	EPA MRID	43334715
	EPA Guideline	72-2

Test material: AMPA **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 **Signature:**
Staff Scientist, Cambridge Environmental Inc. **Date:** 1/22/07

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental Inc. **Date:** 2/21/07

Primary Reviewer: Stephen Carey **Date:** 7/18/07
EPA Biologist, OPP, EFED, ERBIII

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

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 18 2007

CITATION: Burgess, D and S.L. Hicks. 1994. Acute Toxicity of AMPA to *Daphnia magna*. Unpublished study performed by ABC Laboratories, Inc., Columbia, Missouri. Laboratory report number 38988. Study sponsored by Monsanto Agricultural Company, St. Louis, Missouri. Study submitted on May 10, 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 freshwater invertebrates. 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 48-hour acute toxicity of AMPA to *Daphnia magna* was studied under static conditions. Daphnids were exposed to nominal concentrations of 0 (negative control), 100, 180, 320, 560 and 1000 mg ai/L for 48 hr; mean-measured concentrations were 0 (negative control), 97.7, 177, 320, 553 and 1010 mg ai/L. Immobility and sub-lethal effects were observed daily. The 48-hour LC_{50} was 683 mg ai/L. As no quantitative sub-lethal measurements were taken, the reviewer was unable to determine an EC_{50} value. The 48-hr NOAEC, based on immobility and sub-lethal effects, was 320 mg ai/L. The sub-lethal effects included were lying on the bottom of the test vessel and trailing extraneous material.

Based on the results of this study, AMPA would be classified as practically non-toxic to *Daphnia magna* in accordance with the classification system of the U.S. EPA.

This study is classified as scientifically sound and does satisfy guideline requirements for an acute toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age (e.g., 1st instar): <24 Hours
Test Type (Flow-through, Static, Static Renewal): Static

Immobility (mortality)
EC_{50}: 683 mg a..i./L 95% C.I.: 553-1010 mg ai/L
NOAEC: 320 mg a.i./L Probit Slope: N/A

Sub-lethal Effects
LOAEC: 553 mg ai/L
NOAEC: 320 mg ai/L

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

I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in "Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians," Committee on Methods for Toxicity Tests with Aquatic Organisms, Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009. The following deviations from OPPTS 850.1010 were noted:

1. The physiochemical properties of the test material were not reported.
2. The reported hardness of the dilution water (160 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The reported pH values (5.2-8.3) ranged outside of the recommended limits (7.2-7.6) and the pH values tended to decrease with increasing concentration. Furthermore, the chlorine content of the dilution water was not reported.

These deviations do not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. The biological portion of the this study was conducted in compliance with the Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 160 as set down in the Federal Register, Vol. 54, 34052-34074, 17 August 1989, except as may be noted by the Study Director. The analytical portion of this study was conducted by the Sponsor and was not conducted under any current GLP standards.

A. MATERIALS:

1. Test material AMPA

Description: White Powder

Lot No./Batch No. : HET-9001-1463T

Purity: 94.38%

Stability of compound under test conditions: Samples of the dilution water were taken at 0 and 48 hours and analyzed for the content of the test material. Mean-measured concentrations yielded recoveries of 98-101% of nominal. Quality control samples yielded recoveries of 96.7-101% 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.

Physicochemical properties of AMPA.

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

2. Test organism:

Species: *Daphnia magna*
Age at test initiation: <24 Hours
Source: In-house laboratory culture

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 38 hour range-finding was conducted using ten daphnids in exposure concentrations of 1.0, 10 and 100 mg/L. At test termination, no morality or abnormal effects were observed at the 10 and 100 mg/L treatment levels and one daphnid in the 1.0 mg/L treatment group was lying on the bottom of the test vessel. This was not considered treatment-related based on the lack of effects at the other two treatment levels. These results were used to determine the nominal concentrations for the definitive test.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
<u>Acclimation</u> Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 Continuous Same as test Daphnids were fed a suspension of at least one algae species: *Selenastrum capricornutum, Ankistrodesmus falcatus* and/or *Chlamydomonas reinhardrii*. Along with the algae, the daphnids were fed a supplement consisting of Rangens® trout chow and Fleischmann's® active dry yeast. The daphnids were fed this feeding regimen at least every three days. All daphnids appeared normal and healthy	 *The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study. Pretest mortality should be <3% 48 hours prior to testing.*
Duration of the test	48 Hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
<u>Test condition</u> Static/flow-through Type of dilution system for flow-through method. Renewal rate for static renewal	 Static N/A N/A	*The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*
Aeration, if any	No aeration was reported	

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 250 mL 200 mL	 *EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill*
Source of dilution water	The hard blended water was a combination of well water and RO water blended to a hardness of 160-180 mg/L as $CaCO_3$.	*Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated 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 (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010Opdf). Dilution water should be intensely aerated before the study.*

Parameter	Details	Remarks *Criteria*
<u>Water parameters</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 160 mg/L as $CaCO_3$ 5.2-8.3 8.5-8.7 mg/L 19-20^oC <1.0 mg/L (October 1990) 1.4 mg/L (TSS; October 1990) See Reviewer's Comments None Detected Not Reported	The DO values represented 94-101% saturation at 19 and 20^oC after correcting for the altitude of the testing laboratory. *<u>Hardness</u>:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 140 - 250 mg/L)* *<u>pH:</u>* *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations* *<u>Temperature</u>:* *EPA recommends: 20^oC for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1^oC;* OECD *recommends* range of 18-22^oC (±1^oC) *<u>Dissolved oxygen</u>:* *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*
<u>Number of replicates</u> Negative control: Treatments:	 2 2	*EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels* *OECD recommends 4 groups of 5 animals for each test concentration and the controls*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Negative control: Treatments:	 10 10	*EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the LC_{50}/EC_{50} is >100 mg/L by exposing ∃30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and #0.5 g/L at > 17°C; flow-through: #10 g/L at ≤ 17^B C and ≤5 g/L at > 17^B C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*
Treatment concentrations Nominal: Measured:	 0 (negative control), 100, 180, 320, 560 and 1000 mg ai/L 0 (negative control), 97.7, 177, 320, 553 and 1010 mg ai/L	*Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g/L, while the lowest concentration should have no observable effect.*
Solvent (type, percentage, if used)	N/A; a solvent was not used.	*Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests. OECD recommends that the solvent not exceed 100 mg/L.*

Parameter	Details	Remarks *Criteria*
Lighting	16L:8D	Lighting was maintained at 37-74 footcandles with 30 minute simulated dawn and dusk periods. *EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD: optional light-dark cycle or complete darkness.*
Stability of chemical in the test system	Stable. Mean-measured concentrations yielded recoveries of 98-101% of nominal.	
Recovery of chemical Level of Quantitation Level of Detection	 Not Reported Not Reported	Quality control samples, analyzed concurrently with the samples of dilution water yielded a recovery of 96.7-101% of nominal.
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

Criteria	Details	Remarks
Parameters measured including the sublethal effects	Immobilization/mortality and sub-lethal effects	
Observation intervals	24 and 48 hours	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. IMMOBILITY / MORTALITY:

By test termination (48 hours) immobility was 5% in the negative control and 0, 0, 0, 15 and 100% in the mean-measured 97.7, 177, 320, 553 and 1010 mg ai/L treatment groups, respectively. Immobility first occurred after 24 hours of exposure in the mean-measured 1010 mg ai/L treatment group. The EC_{50} value (and 95% C.I.) was 690 (560-1000) mg ai/L as determined by the study authors using the binomial method; the reported NOAEC value was 320 mg ai/L.

Table 3: Effect of AMPA on Immobility of *Daphnia* sp.

Treatment (mg a.i./L) Mean-Measured and (Nominal)	No. of organisms	Observation Period			
		Day 1		Day 2	
		No Dead / Immobilized	% Immobility	No Dead / Immobilized	% Immobility
Negative Control	20	0	0	1	5
97.7 (100)	20	0	0	0	0
177 (180)	20	0	0	0	0
320 (320)	20	0	0	0	0
553 (560)	20	0	0	3	15
1010 (1000)	20	17	85	20	100
NOAEC	320 mg ai/L*				
EC_{50}	690 (560-1000) mg ai/L*				
Positive control, if used Mortality: LC_{50} NOAEC:	N/A	N/A	N/A	N/A	N/A

N/A- Not Applicable
*- Values based on nominal concentrations

B. SUB-LETHAL TOXICITY ENDPOINTS:

After 24 hours of exposure, one daphnid in the negative control and one daphnid in the mean-measured 553 mg ai/L treatment group were on the bottom of the test vessel; however, by test termination, all daphnids in the negative control and mean-measured 97.7-320 mg ai/L treatment groups appeared normal. Two of the surviving daphnids in the mean-measured 553 mg ai/L treatment group were exhibiting sub-lethal effects at test termination; one was surfacing and one was trailing extraneous material. The NOAEC and LOAEC values, based on sub-lethal effects, were 320 and 553 mg ai/L, respectively (320 and 560 mg ai/L, respectively, based on nominal concentrations).

Table 4: Effect of AMPA on Sub-lethal Endpoints - *Daphnia* sp.

Treatment (mg a.i./L) Mean-Measured and (Nominal)	Observation Period			
	Day 1		Day 2	
	Endpoints	% Affected	Endpoints	% Affected
Negative Control	On bottom of test vessel	10%	A.N.	0
97.7 (100)	A.N.	0	A.N.	0
177 (180)	A.N.	0	A.N.	0
320 (320)	A.N.	0	A.N.	0
553 (560)	On bottom of test vessel	10%	Surfacing Trailing extraneous material	5.8% 5.8%
1010 (1000)	A.N.	0	--	--
NOAEC	320 mg ai/L*			
LOAEC	560 mg ai/L*			
EC_{50}	Not Determined			
Positive control, if used % sublethal effect: EC_{50}	N/A	N/A	N/A	N/A

N/A- Not Applicable
-- Complete Immobility/Mortality
*- Values based on nominal concentrations

C. REPORTED STATISTICS:

Statistical analysis of the concentration vs. effect data (mortality/immobility) was attempted by employing a computerized LC_{50}/EC_{50} program developed by Stephen et al. The binomial method was used to determine the 48-hour EC_{50} value and the NOAEC value was determined visually.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The 48-hour EC_{50} (and 95% C.I.) was determined using the binomial method via Toxanal statistical software. The NOAEC value, based on mortality/immobility, was determined using Fisher's Exact Test via Toxstat statistical software. The NOAEC value, based on sub-lethal effects, was determined visually. As no quantitative sub-lethal measurements were taken, the reviewer was unable to determine an EC_{50} value. All toxicity values were determined using the mean-measured concentrations.

Immobility (mortality)
EC_{50}: 683 mg a..i./L 95% C.I.: 553-1010 mg ai/L
NOAEC: 320 mg a.i./L Probit Slope: N/A

Sub-lethal Effects

LOAEC: 553 mg ai/L
NOAEC: 320 mg ai/L

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results were based on the mean-measured concentrations, while those of the study authors were based on nominal concentrations. Therefore, the reviewer's results 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 43334716. Holland, M.E. 1994. Results of the Analyses of AMPA in a 48-Hour Acute Study with *Daphnia magna*. Guideline 72-2.

The results from the periodic screening analysis of the dilution water indicated the presence of the following elements: boron (0.582 mg/L), copper (0.033 mg/L), fluoride (0.98 mg/L), iron (0.332 mg/L), lead (12.2 mg/L) and zinc (0.067 mg/L).

The in-life portion of the definitive toxicity test was conducted from November 24 to November 26, 1990.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. The 48 hour EC_{50}, NOAEC, and LOAEC values were 683, 320 and 553 mg ai/L, respectively.

Immobility (mortality)
EC_{50}: 683 mg a..i./L 95% C.I.: 553-1010 mg ai/L
NOAEC: 320 mg a.i./L Probit Slope: N/A

Sub-lethal Effects
LOAEC: 553 mg ai/L
NOAEC: 320 mg ai/L

III. REFERENCES:

Personal Communication, Herman O. Sanders, 1979, United States Department of the Interior.

Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W. Andrews. 1978. A Computer Program for Calculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, MN, pre-publication manuscript, August, 1978.

Stephen, C.E. 1977. Methods for Calculating an LC_{50}, p. 65-84. In F.L. Mayer and J.L. Hamelink (eds). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009.

U.S. Environmental Protection Agency. 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (49 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.

Japanese Ministry of Agriculture, Forestry and Fisheries. 1984. Good Laboratory Practice Standards; 59 Nohsan No. 3850.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER EXPOSED	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1010	20	20	100	9.536742E-05
553	20	3	15	.1288414
320	20	0	0	9.536742E-05
177	20	0	0	9.536742E-05
97.7	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 553 AND 1010 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 683.3248

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	1	
1	97.7	20	0	
2	177	20	0	
3	320	20	0	
4	**553**	**20**	**3**	
5	1010	20	20	*