DP Barcode : D220177
PC Code No : 069105
EEB Out :
MAR 20 1996

To: Beverly Lavis (PM 72)
Product Manager
Special Review and Reregistration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 069105
Chemical Name : ADBAC
Type Product : Biocide
Product Name : ADBAC
Company Name : Chemical Specialties Manufacturers Association
Purpose : Review of three 96hr Acute LC50 studies for toxicity of ADBAC to Fathead Minnows

Action Code : 627 Date Due :
Reviewer : Harry A. Winnik Date In EEB: 10/27/95

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)	437401-01 437401-02 437401-03	S S C	72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

DP BARCODE: D220177 REREG CASE # 0350

CASE: 819070 DATA PACKAGE RECORD DATE: 10/18/95
SUBMISSION: S495478 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 627 CORE DATA
CHEMICALS: 069105 Alkyl* dimethyl benzyl ammonium chloride *(50%C14, 100.00 %

ID#: 069105
COMPANY:
PRODUCT MANAGER: 72 LARRY SCHNAUBELT 703-308-8058 ROOM: CS1 3E3
PM TEAM REVIEWER: BEVERLY LAVIS 703-308-8376 ROOM: CS1 3RD FL
RECEIVED DATE: 07/25/95 DUE OUT DATE: 10/23/95

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 220177 EXPEDITE: N DATE SENT: 10/18/95 DATE RET.: / /
CHEMICAL: 069105 Alkyl* dimethyl benzyl ammonium chloride *(50%C14, 40%C12,
DP TYPE: 001 Submission Related Data Package
CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	10/18/95	/ /
BRAN: EEB	10/27/95	/ /
SECT: IV	10/27/95	/ /
REVR : Schmitt	10/27/95	/ /
CONTR:	/ /	/ /

ADMIN DUE DATE: 01/16/96
NEGOT DATE: / /
PROJ DATE: / /

* * * DATA REVIEW INSTRUCTIONS * * *

Please review the attached fathead minnow studies GLN 72-1a, 43740101, 43740102, 43740103. Call Beverly Lavis @ 308-8376 for questions or concerns.

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

Beverly Lavis @ 703-308-8376

Lavis.Beverly@epamail.epa.gov

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

March 15, 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Acute Fathead Minnow Studies with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC)

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Beverly Lavis (PM 72)
Reregistration Division

EEB has completed review of three acute toxicity studies submitted by the Chemical Specialties Manufacturers Association to support the reregistration of ADBAC (copies attached). The following is a brief summary of the reviews:

CITATION: Sword, Marc C., & Luke Stuerman, 1993, Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*), prepared by ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202, submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, ABC Laboratories' Study #41237, 437401-03

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a), and can be classified as **Core**. The 96-hour LC_{50} value was 0.28 ppm mean measured concentration. Therefore, ADBAC is classified as highly toxic to fathead minnows. Since there was mortality at all concentrations tested, no NOEC value was available.

CITATION: Sword, Marc C., & Luke Stuerman, 1993, Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*) in Dilution Water Amended with 10 mg/L Humic Acid, prepared by ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202, submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, ABC Laboratories' Study #41236, 437401-02

CONCLUSIONS: This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity

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test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water and can be classified as **Supplemental.** The 96-hour LC_{50} value was 0.77 ppm mean measured concentration. Therefore, in the presence of 10 mg/L humic acid, ADBAC is considered highly toxic to fathead minnows. The NOEC was 0.53 ppm.

CITATION: Sword, Marc C., & Luke Stuerman, 1993, Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*) in Dilution Water Amended with 20 mg/L Humic Acid, prepared by ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202, submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, ABC Laboratories' Study #41235, 437401-01

CONCLUSIONS: This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water and can be classified as **Supplemental.** The 96-hour LC_{50} value was 1.4 ppm mean measured concentration. Therefore, in the presence of 20 mg/L humic acid, ADBAC is considered moderately toxic to fathead minnows. The NOEC was 0.99 ppm.

If you have any questions regarding this document please contact Harry A. Winnik, Biologist, 305-7089.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

March 15, 1996

MEMORANDUM

SUBJECT: Review of Acute Fathead Minnow Studies with Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC)

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Beverly Lavis (PM 72)
Reregistration Division

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CITATION: Sword, Marc C., & Luke Stuerman, 1993, Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*), prepared by ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202, submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, ABC Laboratories' Study #41237, 437401-03

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a), and can be classified as **Core**. The 96-hour LC_{50} value was 0.28 ppm mean measured concentration. Therefore, ADBAC is classified as highly toxic to fathead minnows. Since there was mortality at all concentrations tested, no NOEC value was available.

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CONCLUSIONS: This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity

CONCURRENCES

SYMBOL	7507C	7507C	7507C					
SURNAME	WINNIK	Craven	Lois Rinden					
DATE	3/18/96	3/18/96	03/20/96					

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test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water and can be classified as **Supplemental**. The 96-hour LC_{50} value was 0.77 ppm mean measured concentration. Therefore, in the presence of 10 mg/L humic acid, ADBAC is considered highly toxic to fathead minnows. The NOEC was 0.53 ppm.

CITATION: Sword, Marc C., & Luke Stuerman, 1993, Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*) in Dilution Water Amended with 20 mg/L Humic Acid, prepared by ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202, submitted by ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association, ABC Laboratories' Study #41235, 437401-01

CONCLUSIONS: This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water and can be classified as **Supplemental**. The 96-hour LC_{50} value was 1.4 ppm mean measured concentration. Therefore, in the presence of 20 mg/L humic acid, ADBAC is considered moderately toxic to fathead minnows. The NOEC was 0.99 ppm.

If you have any questions regarding this document please contact Harry A. Winnik, Biologist, 305-7089.

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DATA EVALUATION RECORD
§ 72-1(A) -- ACUTE LC_{50} TEST WITH A WARMWATER FISH

1. **CHEMICAL:** Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) **PC Code No.:** 069105

2. **TEST MATERIAL:** ADBAC Quat 80% (lot #7293k) **Purity:** 81.9 %
^{14}C-ADBAC (ABC Ref. #RS-6654 **Purity:** 98.4%

3. **CITATION**

Authors:	Sword, Marc C., & Luke Stuerman
Title:	Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*)
Study Completion Date:	November 19, 1993
Laboratory:	ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202
Sponsor:	ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association
Laboratory Report ID:	ABC Laboratories' Study #41237
MRID No.:	437401-03
DP Barcode:	D220177

4. **REVIEWED BY:** Harry A. Winnik, Biologist, EEB, EFED

Signature: **Date:** 3/18/96

5. **APPROVED BY:** Henry T. Craven, Head of Section #IV, EEB, EFED

Signature: Henry T. Craven **Date:** 3/18/96

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	Fathead Minnow (*Pimephales promelas*)
Age or Size of Test Organism:	Mean length: 18 ± 3mm Range: 15 - 26mm Weight: 0.08 ± 0.05 g
Definitive Test Duration:	96 hours
Study Method:	Static-Renewal
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**

Results Synopsis
LC_{50}: 0.28 ppm ai 95% C.I.: 0.23-0.34 ppm ai
NOEC: N/A Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

B. Rationale: This study is scientifically sound and fulfills the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a)

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS**

There were no major guideline deviations in this study.

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10. **SUBMISSION PURPOSE:** This study was submitted by the ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association to support the reregistration of ADBAC.

11. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species Preferred species is the bluegill sunfish (*Lepomis macrochirus*)	Fathead Minnow (*Pimaphales promelas*)
Mean Weight 0.5-5 g	0.08 ± 0.05 g
Mean Standard Length Longest not > 2x shortest	Mean: 18 ± 3mm Range: 15 - 26mm
Supplier	ABC Laboratories in-house culture in Columbia, Missouri
All fish from same source?	Yes
All fish from the same year class?	Not reported

B. **Source/Acclimation**

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	2 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	Last feeding was 48 hrs prior to start of the study
Pretest Mortality No more than 3% mortality 48 hours prior to testing	0% mortality prior to testing.

C. **Test System**

Guideline Criteria	Reported Information

Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Blend of naturally hard well water with demineralized well water
Does water support test animals without observable signs of stress?	Yes
Water Temperature 17°C or 22°C	22 ±1°C
pH Prefer 7.2 to 7.6	7.9-8.3
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	7.7-8.1 mg/L (91.9%-96.6%)
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	130-160 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	1. Material: Glass . 2. Size: 5 gal. 3. Fill volume: 15L of solution.
Type of Dilution System Must provide reproducible supply of toxicant	N/A
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	volumes renewed every 24 hours
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.05g/L
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark with 30 transition periods
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: None used

D. Test Design

Guideline Criteria	Reported Information

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Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	two range finding tests were performed and one definitive test was begun and abandoned prior to final test
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	control 0.10 mg ai/L. 0.18 mg ai/L. 0.32 mg ai/L. 0.56 mg ai/L. 1.00 mg ai/L.
Number of Test Organisms Minimum 10/level, may be divided among containers	20 fish per treatment level (2 replicates per treatment level containing 10 fish each)
Test organisms randomly or impartially assigned to test vessels?	Yes.
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	"Temperature, dissolved oxygen, and pH were measured in all new control and test solutions at 0-, 24-, 48-, and 72-hr of testing and on all old control and test solutions at 24-, 48-, 72-, and 96-hr of testing
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	The concentrations of ADBAC were measured at 0-hr of new solutions and at 0-, 24-, 48-, 72-, and 96-hr for old solutions. The mean measured concentrations were as follows: 0 mg ai/L. 0.096 mg ai/L. 0.18 mg ai/L. 0.31 mg ai/L. 0.57 mg ai/L. 1.0 mg ai/L.

12. **REPORTED RESULTS**

A. **General Results**

Guideline Criteria	Reported Information

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Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	96-102%
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	0	20	0	0	0	0
0.10	0.096	20	0	0	1	1
0.18	0.18	20	0	1	4	4
0.32	0.31	20	2	5	6	6
0.56	0.57	20	20	20	20	20
1.0	1.0	20	20	20	20	20

Other Significant Results:

B. Statistical Results

Method: Moving Average Method

96-hr LC_{50}: 0.28 ppm ai 95% C.I.: 0.23-0.34 ppm ai

Probit Slope: N/A_ NOEC: N/A

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	0.355(0.18-0.57) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	0.28 (0.23-0.34) ppm ai
Probit LC_{50} (95% C.I.)	0.30 (0.35-8.68) ppm ai*

Probit Slope	4.52
NOEC	N/A

*Since the probability is less than 0.05, results calculated using the probit method probably should not be used.

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a), and can be classified as **Core**. The 96-hour LC_{50} value was 0.28 ppm mean measured concentration. Therefore, ADBAC is classified as highly toxic to fathead minnows. Since there was mortality at all concentrations tested, no NOEC value was available.

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1	20	20	100	9.536742E-05
.57	20	20	100	9.536742E-05
.31	20	6	30	5.765915
.18	20	4	20	.5908966
.096	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT .18 AND .57 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 .355454

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	5.984823E-02	.2789576	.226231	.3388533

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.8492608	3.004995	2.909225E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.515437
95 PERCENT CONFIDENCE LIMITS = .3542204 AND 8.676653

LC50 = .2961794
95 PERCENT CONFIDENCE LIMITS = 7.970793E-02 AND 1.055912

LC10 = .1549878
95 PERCENT CONFIDENCE LIMITS = 6.00831E-05 AND .2598168

****

USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

DATA EVALUATION RECORD
§ 72-1(A) -- ACUTE LC_{50} TEST WITH A WARMWATER FISH

1. CHEMICAL: Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) PC Code No.: 069105
2. TEST MATERIAL: ADBAC Quat 80% (lot #7293k) Purity: 81.9 %
^{14}C-ADBAC (ABC Ref. #RS-6654 Purity: 98.4%
3. CITATION

Authors: Sword, Marc C., & Luke Stuerman
Title: Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*) in Dilution Water Amended with 20 mg/L Humic Acid
Study Completion Date: December 3, 1993
Laboratory: ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202
Sponsor: ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association
Laboratory Report ID: ABC Laboratories' Study #41235
MRID No.: 437401-01
DP Barcode: D220177

4. REVIEWED BY: Harry A. Winnik, Biologist, EEB, EFED
Signature: [signature] Date: 3-15-96
5. APPROVED BY: Henry T. Craven, Head of Section #IV, EEB, EFED
Signature: Henry T. Craven Date: 3/18/96
6. STUDY PARAMETERS

Scientific Name of Test Organism:	Fathead Minnow (*Pimephales promelas*)
Age or Size of Test Organism:	Mean length: 21 ± 3mm Range: 16 - 26mm Weight: 0.13 ± 0.05 g
Definitive Test Duration:	96 hours
Study Method:	Static-Renewal
Type of Concentrations:	Mean measured

7. CONCLUSIONS: In the presence of 20 mg/L humic acid, ADBAC is considered moderately toxic to fathead minnows.

Results Synopsis
LC_{50}: 1.4 ppm ai 95% C.I.: 0.99-1.8 ppm ai
NOEC: 0.99 ppm ai Probit Slope: N/A

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

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B. **Rationale:** This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water.

C. **Repairability:** This study does not need to be repeated

9. **GUIDELINE DEVIATIONS**

1. Humic acid was added to the dilution water.

10. **SUBMISSION PURPOSE:** This study was submitted by the ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association to support the reregistration of ADBAC.

11. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species Preferred species is the bluegill sunfish (*Lepomis macrochirus*)	Fathead Minnow (*Pimaphales promelas*)
Mean Weight 0.5-5 g	0.13 ± 0.05 g
Mean Standard Length Longest not > 2x shortest	Mean: 21 ± 3mm Range: 16 - 26mm
Supplier	ABC Laboratories in-house culture in Columbia, Missouri
All fish from same source?	Yes
All fish from the same year class?	Not reported

B. **Source/Acclimation**

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	2 days

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Guideline Criteria	Reported Information
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	Last feeding was 48 hrs prior to start of the study
Pretest Mortality No more than 3% mortality 48 hours prior to testing	0% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Blend of naturally hard well water with demineralized well water
Does water support test animals without observable signs of stress?	Yes
Water Temperature 17þC or 22þC	22 ±1þC
pH Prefer 7.2 to 7.6	7.8-8.0
Dissolved Oxygen Static: þ 60% during 1st 48 hrs and þ 40% during 2nd 48 hrs, flow-through: þ 60%	7.5-8.4 mg/L (89.0%-102%)
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	146-148 mg/L as $CaCO_3$

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	1. Material: Glass . 2. Size: 5 gal. 3. Fill volume: 15L of solution.
Type of Dilution System Must provide reproducible supply of toxicant	N/A
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: þ 0.8 g/L at þ 17þC, þ 0.5 g/L at > 17þC; flow-through: þ 1 g/L/day	0.09g/L
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark with 30 transition periods
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: None used However, 20,mg/L humic acid was added to all treatment containers and a humic acid control was included.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	two range finding tests were performed and one definitive test was begun and abandoned prior to final test

18

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	control humic acid control (10mg/L) 0.32 mg ai/L. with humic acid 0.56 mg ai/L. with humic acid 1.0 mg ai/L. with humic acid 1.8 mg ai/L. with humic acid 3.2 mg ai/L. with humic acid
Number of Test Organisms Minimum 10/level, may be divided among containers	20 fish per treatment level (2 replicates per treatment level containing 10 fish each)
Test organisms randomly or impartially assigned to test vessels?	Yes.
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1pC 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	"Temperature, dissolved oxygen, and pH were measured in all new control and test solutions at 0-, 24-, 48-, and 72-hr of testing and on all old control and test solutions at 24-, 48-, 72-, and 96-hr of testing
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	The concentrations of ADBAC were measured at 0-hr of new solutions and at 0-, 24-, 48-, 72-, and 96-hr for old solutions. The mean measured concentrations were as follows: control 0 mg ai/L. humic acid control 0 mg ai/L 0.30 mg ai/L. 0.54 mg ai/L. 0.99 mg ai/L. 1.8 mg ai/L. 3.2 mg ai/L.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	94-100%
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	0	20	0	0	0	0
Humic Acid control	0	20	0	0	0	0
0.32	0.30	20	0	0	0	0
0.56	0.54	20	0	0	0	0
1.0	0.99	20	0	0	0	0
1.8	1.8	20	19	19	19	19
3.2	3.2	20	20	20	20	20

Other Significant Results:

B. Statistical Results

Method: Binomial Test

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96-hr LC_{50}: 1.4 ppm ai 95% C.I.: 0.99-1.8 ppm ai

Probit Slope: N/A_ NOEC: 0.99 ppm ai

13. **VERIFICATION OF STATISTICAL RESULTS**

Parameter	Result
Binomial Test LC_{50} (C.I.)	1.4(0.99-1.8) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	0.99 ppm ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water and can be classified as **Supplemental.** The 96-hour LC_{50} value was 1.4 ppm mean measured concentration. Therefore, in the presence of 20 mg/L humic acid, ADBAC is considered moderately toxic to fathead minnows. The NOEC was 0.99 ppm.

HARRY A. WINNIK ACUTE FATHEAD LD50 +20MG/L HUMIC ACID ADBAC

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.2	20	20	100	9.536742E-05
1.8	20	19	95	2.002716E-03
.99	20	0	0	9.536742E-05
.54	20	0	0	9.536742E-05
.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .99 AND 1.8 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 1.388385

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.

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DATA EVALUATION RECORD
§ 72-1(A) -- ACUTE LC_{50} TEST WITH A WARMWATER FISH

1. **CHEMICAL:** Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) **PC Code No.:** 069105

2. **TEST MATERIAL:** ADBAC Quat 80% (lot #7293k) **Purity:** 81.9 %
^{14}C-ADBAC (ABC Ref. #RS-6654 **Purity:** 98.4%

3. **CITATION**

Authors: Sword, Marc C., & Luke Stuerman
Title: Static-Renewal Acute Toxicity of ADBAC to Fathead Minnow (*Pimephales promelas*) in Dilution Water Amended with 10 mg/L Humic Acid
Study Completion Date: November 19, 1993
Laboratory: ABC Laboratories, Inc., Environmental Toxicology Division, 7200 E. ABC Lane, Columbia, Missouri 65202
Sponsor: ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association
Laboratory Report ID: ABC Laboratories' Study #41236
MRID No.: 437401-02
DP Barcode: D220177

4. **REVIEWED BY:** Harry A. Winnik, Biologist, EEB, EFED

Signature: [signature] **Date:** 3-15-96

5. **APPROVED BY:** Henry T. Craven, Head of Section #IV, EEB, EFED

Signature: Henry T. Craven **Date:** 3/18/96

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	Fathead Minnow (*Pimephales promelas*)
Age or Size of Test Organism:	Mean length: 19 ± 3mm Range: .15 - 25mm Weight: 0.1 ± 0.05 g
Definitive Test Duration:	96 hours
Study Method:	Static-Renewal
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** In the presence of 10 mg/L humic acid, ADBAC is considered highly toxic to fathead minnows.

Results Synopsis

LC_{50}: 0.77 ppm ai 95% C.I.: 0.53-0.98 ppm ai
NOEC: 0.53 ppm ai Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

A. Classification: Supplemental

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B. **Rationale:** This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water.

C. **Repairability:** This study does not need to be repeated

9. **GUIDELINE DEVIATIONS**

1. Humic acid was added to the dilution water.

10. **SUBMISSION PURPOSE:** This study was submitted by the ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Association to support the reregistration of ADBAC.

11. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species Preferred species is the bluegill sunfish (*Lepomis macrochirus*)	Fathead Minnow (*Pimaphales promelas*)
Mean Weight 0.5-5 g	0.10 ± 0.05 g
Mean Standard Length Longest not > 2x shortest	Mean: 19 ± 3mm Range: 15 - 25mm
Supplier	ABC Laboratories in-house culture in Columbia, Missouri
All fish from same source?	Yes
All fish from the same year class?	Not reported

B. **Source/Acclimation**

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	2 days

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Guideline Criteria	Reported Information
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	Last feeding was 48 hrs prior to start of the study
Pretest Mortality No more than 3% mortality 48 hours prior to testing	0% mortality prior to testing.

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Blend of naturally hard well water with demineralized well water
Does water support test animals without observable signs of stress?	Yes
Water Temperature 17þC or 22þC	22 ±1þC
pH Prefer 7.2 to 7.6	7.6-8.2
Dissolved Oxygen Static: þ 60% during 1st 48 hrs and þ 40% during 2nd 48 hrs, flow-through: þ 60%	8.1-8.3 mg/L (96.6%-97.3%)
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	130-160 mg/L as $CaCO_3$

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	1. Material: Glass . 2. Size: 5 gal. 3. Fill volume: 15L of solution.
Type of Dilution System Must provide reproducible supply of toxicant	N/A
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: þ 0.8 g/L at þ 17þC, þ 0.5 g/L at > 17þC; flow-through: þ 1 g/L/day	0.07g/L
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark with 30 transition periods
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: None used However, 10,mg/L humic acid was added to all treatment containers and a humic acid control was included.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	two range finding tests were performed and one definitive test was begun and abandoned prior to final test

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Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	control humic acid control (10mg/L) 0.32 mg ai/L. with humic acid 0.56 mg ai/L. with humic acid 1.0 mg ai/L. with humic acid 1.8 mg ai/L. with humic acid 3.2 mg ai/L. with humic acid
Number of Test Organisms Minimum 10/level, may be divided among containers	20 fish per treatment level (2 replicates per treatment level containing 10 fish each)
Test organisms randomly or impartially assigned to test vessels?	Yes.
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1ÞC 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	"Temperature, dissolved oxygen, and pH were measured in all new control and test solutions at 0-, 24-, 48-, and 72-hr of testing and on all old control and test solutions at 24-, 48-, 72-, and 96-hr of testing
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	The concentrations of ADBAC were measured at 0-hr of new solutions and at 0-, 24-, 48-, 72-, and 96-hr for old solutions. The mean measured concentrations were as follows: control 0 mg ai/L. humic acid control 0 mg ai/L 0.30 mg ai/L. 0.53 mg ai/L. 0.98 mg ai/L. 1.8 mg ai/L. 3.2 mg ai/L.

12. REPORTED RESULTS

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A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	94-100%
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	0	20	0	0	0	0
Humic Acid control	0	20	0	0	0	0
0.32	0.30	20	0	0	0	0
0.56	0.53	20	0	0	0	0
1.0	0.98	20	16	16	17	18
1.8	1.8	20	20	20	20	20
3.2	3.2	20	20	20	20	20

Other Significant Results:

B. Statistical Results

Method: Binomial Test

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96-hr LC_{50}: 0.77 ppm ai 95% C.I.: 0.53-0.98 ppm ai

Probit Slope: N/A_ NOEC: 0.53 ppm ai

13. **VERIFICATION OF STATISTICAL RESULTS**

Parameter	Result
Binomial Test LC_{50} (C.I.)	0.77(0.53-0.98) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	0.53 ppm ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and but does not fulfill the guideline requirements for an acute fish toxicity test of ADBAC with warmwater fish (Guideling 72-1(a)) due to the addition of humic acid to the dilution water and can be classified as **Supplemental.** The 96-hour LC_{50} value was 0.77 ppm mean measured concentration. Therefore, in the presence of 10 mg/L humic acid, ADBAC is considered highly toxic to fathead minnows. The NOEC was 0.53 ppm.

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HARRY A. WINNIK ADBAC ACUTE FATHEAD WITH 10% HUMIC ACID

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.2	20	20	100	9.536742E-05
1.8	20	20	100	9.536742E-05
.98	20	18	90	2.012253E-02
.53	20	0	0	9.536742E-05
.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .53 AND .98 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOGIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .7703483

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.

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