

US EPA ARCHIVE DOCUMENT

Data Evaluation Record

ACROLEIN

Estuarine Fish Acute Toxicity Test
Guideline Reg. No. 72-3(a)

1. TEST MATERIAL - 2-propenal, Acrolein 94.7% (W/W?)

2. STUDY MATERIAL:

Acrolein-2,3-¹⁴C 85.2% (W/W?)

Water - 3.5%, hydroquinone - 0.21%, acetaldehyde - 0.10%,
Benzene - 0.02%, dimers -1.43%

3. STUDY TYPE:

72-3(A) Estuarine fish acute toxicity.

Species tested- Sheepshead minnow (*Cyprinodon variegatus*)

4. STUDY IDENTIFICATION:

Bettencourt, M.J. 1994. Acrolein - Acute toxicity to Sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions. Performed by Springborn Bionomics, Inc., 790 Main Street, Wareham, MA 02571. SLI Report #94-1-5150, SLI Study # 12167.0292.6105.505. Submitted by Baker Performance Chemicals, Inc., 3920 Essex Lane, Houston, Texas 77227-7714. No submitter's number. D204959. S468986. MRID 432252-02. Guideline 72-3a.

5. REVIEWED BY:

James J. Goodyear, Ph.D.
Biologist, Section 1
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: James J. Goodyear

Date: 9-9-94

6. APPROVED BY:

Leslie W. Touart, Ph.D.
Head, Section 1
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: LT

Date: 9/12/94

7. CONCLUSIONS:

The study was judged to be scientific valid but contained a major flaw and was classified as "Supplemental." The guideline requirement is fulfilled and study need not be repeated. The LC₅₀ was 428 µg ai/l for replicate "A."

8. RECOMMENDATIONS - N/A.

9. BACKGROUND- Submitted for reregistration.

10. DISCUSSION OF INDIVIDUAL TEST - N/A.

11. MATERIALS AND METHODS:

A. TEST CONDITIONS:

Animals - Sheepshead minnows obtained for Aquatic Biosystems, Ft. Collins, CO. They were held in a 500 liter tank and fed a commercial diet for 14 days. Feeding was discontinued 48 hours before the study began. There was no pretest mortality. The fish weighed 0.27 g (CI 0.13 to 0.56 g) and were 26 mm (CI 20 to 33 mm) in length at the beginning of the study. They were not fed during the definitive test.

Containers - Glass aquaria, 39 X 20 X 25 cm, with a standpipe 14.5 cm high were used. This provided a constant volume of 11 liters. The flow was approximately 50 ml/min., which provided 6.5 volumes per 24 hour period.

Solution - The initial sample of Acrolein was dissolved with acetone to make an 82.5% ai solution. A solvent control solution was prepared by adding acetone directly to the dilution water flowing into the exposure vessels. The solvent control solution contained the maximum amount of acetone present in any test concentration (0.5 ml/l). A closed loop recirculating filter system was used with natural seawater.

Salinity - Salinity was measured once per day. It was 32% saline.

Temperature - Temperature was measured once per day in all vessels and continuously in one. It varied between 21 and 22° C.

Duration - 96 Hours, but the Acrolein may have existed for only 24 hours or less in one replicate.

pH - pH was measured once per day, it varied from 7.8 to 7.9.

Dissolved O₂ - O₂ was measured once per day, it varied from 88 to 106% of saturation.

Photoperiod- 16 hours light and 8 hours of dark at 8 to 22 footcandles.

B. DOSE:

The nominal concentrations were 51, 130, 320, 800, and 2,000 µg ai/l. The mean measured concentrations were 46, 130, 270, 680, and 1,400 µg ai/l

C. DESIGN:

The test was conducted with a modified constant flow serial Benoit diluter with a 40% dilution factor. It had five syringe pumps (one for each concentration) and each pump had two syringes (one for each replicate). There were two replicates for each

nominal concentration and the control and solvent control. Each of these glass aquaria was in a temperature control bath.

There were twenty fish per level. Ten fish were "impartially selected" for each vessel. The biological loading was 0.036 g/l

"A 4.0 mg ai/ml stock solution (combination of radiolabeled and nonradiolabeled Acrolein) was prepared." "This stock solution was used in the preparation of Quality Control sample and for diluter stock solution during definitive testing. A second 4.0 mg ai/ml stock solution was prepared for the pretest and 0-Hour use." "A final 4.0 mg ai/ml stock solution prepared at 96 hour of exposure" [¶¶ 1 and 3, page 14]. "Samples were removed from each replicate test solution for each treatment level and the controls at 0 hour and 96 hours of exposure . . . Three Quality Control (QC) samples were prepared at each sampling interval and remained with the set of exposure solution samples through out the analytical process" [¶ 1, page 17]. "All samples were analyzed for [¹⁴C]Acrolein using a liquid scintillation counting (LSC) procedure according to the methodology described in Appendix VI [¶1, page 17].

"In addition, samples of the [¹⁴C]Acrolein diluter stock solution (4.0 mb ai/ml) were removed at each sampling interval for analysis of parent Acrolein concentration. Samples were removed at each sampling interval for analysis of parent Acrolein concentration. Samples were analyzed in triplicate using a high performance liquid chromatographic procedure with a radiometric detector (HPLC-RAM) [¶2, page 17].

D. STATISTICS:

Stephan, *et al.* 1982.

12. REPORTED RESULTS:

LC₅₀ = 570 µg ai/l (CI 470 to 700 µg ai/l).

13. STUDY AUTHORS' CONCLUSIONS/QA MEASURES:

The 96-hour LC₅₀ (moving average) was 570 µg ai/l (CI 470 to 700 µg ai/l). Acrolein would be classified as highly toxic to the Sheepshead minnow.

"The data and report . . . were produced and compiled in accordance with all pertinent WPA Good Laboratory Practice regulations," with minor exceptions.

14. REVIEWER'S DISCUSSION AND CONCLUSIONS:

A. TEST PROCEDURES:

Since the fish weighed less than 0.5 g, they should have been fed up to the beginning of the test. The pH should have been kept between 8.0 to 8.3.

B. STATISTICAL ANALYSIS:

Stephan, C.E. 1977.

$LC_{50} = 579.9 \mu\text{g ai/l}$ (CI 473.8 to 733.5 $\mu\text{g ai/l}$)

OR

24 96 hour $LC_{50} = 428.4 \mu\text{g ai/l}$ (CI 270 to 680 $\mu\text{g ai/l}$) based upon replicate "A" only.

C. DISCUSSION/RESULTS:

Acrolein has a history of being difficult to maintain in a study solution, because of its volatility, hydrolysis, and other reasons. It is necessary to measure it at all stages to determine its level. In this study, the registrant decided to use a flow through design to replace the chemical as it evaporated and degraded.

Many chemicals that have problems in maintaining a constant level of active ingredient combine with the fecal material, bacteria, or other organic matter in the water and become unavailable to the study subjects. The registrant states (letter of May 24, 1993, 12167.6105) that Acrolein has a half life of less than 4 hours.

The concentrations of Acrolein in the solutions were determined indirectly by measuring the scintillations from the [^{14}C]Acrolein. The radioactive portions of the Acrolein molecule would persist while combined with organic material or after the Acrolein molecule had degraded into its hydrolysis products. The scintillation counter cannot distinguish between ^{14}C atoms in a degradate, combined with other organic molecules, or a part of still active Acrolein.

The study report details the removal of samples from the test replicates for analysis with a scintillation counter. It also relates that the samples from the diluter stock were "removed at each sampling interval for analysis of parent Acrolein." That is, they were analyzed chemically to see how much of the Acrolein was left. No such statement is made about the samples from the replicates. In a telephone call to Kathy Cornel and Don Suprenant, Springborn Laboratories (the performing laboratory) confirmed that the Acrolein was not analyzed chemically and that it is not known how much Acrolein was present in the vessels at 96 hours.

In a July 20, 1993 letter to Robert Foster (Springborn Labs), Anthony F. Maciorowski (EEB Branch Chief) expressed concern about "the discrepancy in hydrolysis rate reported (<4 hours in correspondence versus 37 hours in EFGWB data files) . . . [the toxicity] of major metabolites/degradates." These concerns have not been addressed.

Since [^{14}C]Acrolein produces radiolabeled degradates, the stated concentrations can be viewed as the concentrations of the total of Acrolein and its degradates. The toxicity being measured is then the toxicity of the total Acrolein-degradates system.

It is evident that the toxicity system was not available in replicate "B" of the 680 $\mu\text{g ai/l}$ level and was not available at all after 24 hours. If an LC_{50} of 570 $\mu\text{g/l}$ is accepted, Acrolein would be categorized as being "Highly toxic" to estuarine fish on an acute basis.

If only replicate "A" 24-hour data is used the LC_{50} can be recalculated as 428.4 $\mu\text{g ai/l}$ (CI 270 to 680 $\mu\text{g ai/l}$). Since there were no further mortalities, the

96 hour data would give the same result. Acrolein would still be categorized as being "Highly toxic" to estuarine fish on an acute basis.

D. ADEQUACY OF THE STUDY:

Classification - Supplemental.

Rationale - The concentrations of the test solutions were not accurately determined.

Repair - None.

15. COMPLETION OF ONE-LINER FOR STUDY:

Yes, see attached sheet.

16. CBI APPENDIX - N/A.

LITERATURE CITED

Stephan, C.E. 1977. Methods for calculating an LC_{50} . in, Aquatic Toxicology and Hazard Evaluation. ASTM STP 634. F.L. Mayer and J.L. Hamelink, Eds. American Society for Testing and Materials. pp. 65-84.

ECOLOGICAL EFFECTS BRANCH
HISTORY OF ACROLEIN
DATA REQUIREMENTS FOR PESTICIDE REGISTRATION

REVIEWER'S NAME: James J. Goodyear, Ph.D. TELEPHONE: 703-305-7726

DATE: August 14, 1994

GUIDE-LINES	DATA REQUIREMENTS	FULFILLS DATA REQUIREMENTS (Y/N)	STUDIES REVIEWED		
			MRID	DATE	STATUS
71-1(a)	Acute Avian Oral Quail or Duck	N - 19 mg/kg - HT Waiver denied	92001-003 Bigler	1-15-91 9-19-90	Sup 1975 Required
71-1(b)	Acute Avian Oral Quail or Duck/TEP	W - same as TG	Bigler	9-19-90	Waived
71-2(a)	Avian Dietary/Quail	W - volatility	Bigler	"	Waived
71-2(b)	Avian Dietary/Duck	W - volatility	Bigler	"	Waived
71-4(a)	Avian Reproductive/Quail	W - volatility	Bigler	"	Waived
71-4(b)	Avian Reproductive/Duck	W - volatility	Bigler	"	Waived
72-1(a)	Fish Toxicity Bluegill	N - 22 ppb - VHT	415132-01	1-15-91	Supplmntl
72-1(b)	Fish Toxicity Bluegill/TEP	W - same as TG	Bigler	9-19-90	Waived
72-1(c)	Fish Toxicity Rainbow Trout	N - <31 ppb - VHT	415132-02	1-15-91	Supplmntl
72-1(d)	Fish Toxicity Rainbow Trout/Tep	W - same as TG	Bigler	9-19-90	Waived
72-2(a)	Invertebrate Toxicity	N - <31 ppb - VHT	415132-03	1-15-91	Supplmntl
72-2(b)	Invertebrate Toxicity/TEP	W - same as TG	Bigler	9-19-90	Waived
72-3(a)	Estuarine/Marine Toxicity Fish	N - waiver denied	Bigler	"	Required
72-3(b)	Estuarine/Marine Toxicity Mollusk	N - "	Bigler	"	Required
72-3(c)	Estuarine/Marine Toxicity Shrimp	N - "	Bigler	"	Required
72-3(d)	Estuarine/Marine Toxicity Fish/TEP	W - same as TG	Bigler	"	Waived
72-3(e)	Estuarine/Marine Toxicity Mollusk/TEP	W - same as TG	Bigler	"	Waived
72-3(f)	Estuarine/Marine Toxicity Shrimp/TEP	W - same as TG	Bigler	"	Waived
72-4(a)	Early Life Stage Fish	N - waiver denied	Bigler	"	Required
72-4(b)	Life Cycle Aquatic Invertebrate	N - "	Bigler	"	Required
72-5	Life Cycle Fish	R - waiver denied	Bigler	"	Reserved
72-6	Aquatic Organisms Accumulation	Deferred to EFGWB			
122-2	Aquatic Plant Growth	N - waiver denied	Bigler	9-19-90	Required
124-2	Aquatic Plant Growth	N - waiver denied	Bigler	"	Required

N = Data requirement *not* fulfilled, study required

W = Data requirement has been waived

"Basic Six" study

Y = Data requirement fulfilled

X = Not applicable

R = Reserved

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ECOLOGICAL EFFECTS BRANCH

ACROLEIN

REMAINING DATA REQUIREMENTS FOR PESTICIDE REGISTRATION

REVIEWER'S NAME: James J. Goodyear, Ph.D. TELEPHONE: 703-305-7726 DATE: Aug. 14, 1994

GUIDE-LINES	DATA REQUIREMENTS	FULFILLS DATA REQUIREMENTS (Y/N)	STUDIES REVIEWED		
			MRID	DATE	STATUS
71-1(a)	Acute Avian Oral Quail or Duck	N - 19 mg/kg - HT Waiver denied	92001-003	1-15-91	Sup 1975
			Bigler	9-19-90	Required
72-1(a)	Fish Toxicity Bluegill	N - 22 ppb - VHT	415132-01	1-15-91	Supplmntl
72-1(c)	Fish Toxicity Rainbow Trout	N - <31 ppb - VHT	415132-02	1-15-91	Supplmntl
72-2(a)	Invertebrate Toxicity	N - <31 ppb - VHT	415132-03	1-15-91	Supplmntl
72-3(a)	Estuarine/Marine Toxicity Fish	N			Required
72-3(b)	Estuarine/Marine Toxicity Mollusk	N			Required
72-3(c)	Estuarine/Marine Toxicity Shrimp	N			Required
72-4(a)	Early Life Stage Fish	N			Required
72-4(b)	Life Cycle Aquatic Invertebrate	N			Required
72-5	Life Cycle Fish	N			Required
72-6	Aquatic Organisms Accumulation				
122-2	Aquatic Plant Growth	N			Required
124-2	Aquatic Plant Growth	N			Required

N = Data requirement *not* fulfilled, study required

W = Data requirement has been waived

"Basic Six" Study

Y = Data requirement fulfilled

X = Not applicable

R = Reserved

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