

US EPA ARCHIVE DOCUMENT

UNDATED

DATA EVALUATION RECORD

§ 70-1 - Special Test: ACUTE TOXICITY TEST WITH FRESHWATER INVERTEBRATES

1. **CHEMICAL:** (RS)-a-cyano-3-phenoxybenzyl(1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
2. **TEST MATERIAL:** Cypermethrin
Purity: 92.3 %
3. **Study Type:** LC₅₀ Acute Toxicity Test: *Chironomus riparius*; *Hyaella azteca*.
4. **CITATION:**

Rapley, J.H., and Hamer, M.J. 1996. Cyperpethrin: Toxicity to *Chironomus riparius* and *Hyaella azteca*. Zeneca Agrochemicals Report No. RC0002. Performed by Zeneca Agrochemicals Laboratory for the Pyrethroid Working Group (PWG). EPA MRID #440744-01.

5. **REVIEWED BY:**

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7. **STUDY PARAMETERS**

Test Organism: *Chironomus tentans* : First & fourth instar
Hyaella azteca : 7-14 day old larvae

Definitive Test Duration: 48 hours

Study Method: Static

Type of Concentrations: Mean measured/Nominal

8. **CONCLUSIONS:** These studies are scientifically sound but do not meet the requirements in the proposed ASTM guidelines for an acute. This information on sediment toxicity shows that although cypermethrin, with a K_{oc} of 180,000 - 500,000, binds rapidly to suspended particulate and sediment, it is very highly toxic to *Chironomus* and possibly other benthic organisms.

9. **Guideline Deviations**

10. **SUBMISSION PURPOSE:** This study was designed to investigate the toxicity of cypermethrin to first and fourth instar *Chironomus riparius* larvae and 7-10 day old *Hyalella azteca*.

11. **MATERIALS AND METHODS**

A. **Test Organism:** *Chironomus riparius* were obtained from laboratory cultures at Jealott's Hill Research Station. Cultures were maintained in 5 liter plastic aquaria with a bottom layer of silver sand and a water hardness of about 170 mg l⁻¹. The cultures were maintained at 23°C on a 16 hour: 8 hour light:dark photoperiod and fed ground tetra-min *ad libitum*. Under these conditions the life-cycle of *C. riparius* is about 2-3 weeks. First instars were produced by removing egg ropes from the cultures and hatching in plastic weigh trays before counting out for the test. Fourth instar were counted out from established cultures.

B. **Test Organism:** *Hyalella azteca* were obtained from laboratory cultures at the Jealott's Hill research Station. Mixed age cultures were maintained in hard water at 23°C on a 16 hour:8 hour light:dark cycle. Culture vessels were 20 liter glass aquaria containing 10 liters of water. Horsechestnut (*Aesculus hippocastanum*) leaves, were added as a substrate and food. The *H. azteca* were 7 to 14 days old at the start of the test. These organisms were sorted from the culture (those retained on a 250 um sieve) and transferred to a fresh culture vessel for a further 7 days.

C. **Test Water:** Dilution water was prepared by mixing dechlorinated mains water (hardness of 300 mg l⁻¹) with the same amount of deionized (reverse osmosis system) water. The final hardness was 166 mg l⁻¹ CaCO₃.

D. **Test System:**

Test vessels were 250 ml, 600 ml, and 2 liter for testing first and fourth instar *C. riparius* and

7-14 day old *H. azteca*. Single replicates were tested at each concentration, with 20 organisms added to each test vessel. Test vessels were covered throughout the test to prevent evaporation and to maintain a constant temperature ($23 \pm 2^{\circ}$ C under fluorescent lighting (600 lux) on a 16 hour: 8 hour light:dark cycle. Food was administered during the test. Organisms were assessed for mortality (no visible movement) at 48 hours.

A series of nominal test concentrations were prepared with 0.01% acetone. Both solvent and negative controls were also prepared. Stock solutions of the ^{14}C -labelled cypermethrin in acetone were prepared for each test concentration. Test concentrations were as follows:

C. riparius (1st instar) and *H. azteca*: 0.1, 0.05, 0.025, 0.0125, 0.0062, 0.0031, and 0.0016 $\mu\text{g l}^{-1}$.

C. riparius (4th instar): 4, 2, 1, 0.5, and 0.25 $\mu\text{g l}^{-1}$.

E. Statistical Analysis: Estimates of LC_{50} values and 95% confidence intervals were obtained by the technique of iteratively reweighted linear regression on the logit transformation of the percent response on \log_{10} (concentration). Estimation of LC_{50} was performed using an in-house LOGITPC version 1.2.

F. Analytical Methods of test Chemical:

Liquid Scintillation Counting was carried out using an LKB 1217 Rackbeta liquid scintillation counter. Optiphase Safe was the scintillation cocktail used. Each group of samples was preceded by two blank samples in order to calculate background. Subsequent samples were background corrected and net disintegrations per second (DPS) calculated.

Aliquots of each test concentration were taken for analysis at the time of preparation. At 48 hours, after the assessments, samples were taken for analysis from each test vessel. In order to quantify the amount of ^{14}C -cypermethrin present, 200 ml aliquots of test solution were extracted with 5 ml n-hexane and a 3 ml sub-sample of the hexane analysed by LSC (recoveries in the hexane ranged from 83-114%).

Thin layer chromatography was used to determine the purity of the ^{14}C -cypermethrin at the start of the study. samples were applied to silica gel plates and then developed in the solvent systems:

System 1: toluene:hexane:chloroform:acetonitrile (60:20:4:1).

System 2: chloroform:methanol (98:2).

The radioactive areas on the TLC plates were quantified by scanning using a Rita 68000 (Lablogic Isomess 7).

G. Physicochemical Analysis:

1) Dissolved oxygen and pH were measured in each test vessel (0 and 48 hours) using a YSI Model 57 and Radiometer PHM62 meters, respectively. Temperature was recorded throughout the test using a temperature probe connected to a data logger (readings every 30 minutes).

I. Reported Results:

The 48 hour LC50 values (based on mean measured concentrations) for *H. azteca* and first instar *C. riparius* were not significantly different at 0.0053 and 0.0069 $\mu\text{g l}^{-1}$. Fourth instar *C. riparius* were significantly less sensitive; the calculated LC50 was 4.6 $\mu\text{g l}^{-1}$.

14. REVIEWER'S COMMENTS:

Water hardness levels were very high (166 mg l^{-1} as CaCO_3) as compared to the recommended values of 40 - 48 mg l^{-1} as CaCO_3 . Toxicity values were calculated using the Probit method and are as follows: *C. riparis* (1st instar) LC50 = 0.0068 ppb (95% conf. Inter. 0.005 - 0.008 ppb); *C. riparis* (4th instar) LC50 = 4.6 ppb (95% conf. Inter. 1.99 - 298.2 ppb); *H. azteca* LC50 = 0.0054 ppb (0.004 - 0.006 ppb).

Adequacy of the Studies:

1) **Classification:** Core.

2) **Rationale:** NA

3) **Repairability:** NA

Table 1. Measured Cypermethrin Concentrations

Nominal Concentrations (ug l ⁻¹)	Measured Concentration (ug l ⁻¹)	Measured Concentration (ug l ⁻¹)	Average Measured Concentration (ug l ⁻¹)
	0 hr	48 hr	
<i>C. riparius</i> 4th instar			
4.0			
2.0	3.74	2.04	2.89
1.0	1.95	0.886	1.42
0.50	1.01	0.465	0.736
0.20	0.454	0.211	0.332
Control	0.279	0.128	0.204
	<0.0005	<0.0005	<0.0005
<i>C. riparius</i> 1st instar			
0.10			
0.050	0.116	0.0283	0.072
0.025	0.057	0.0172	0.037
0.0125	0.030	0.0078	0.019
0.0062	0.016	0.0037	0.010
0.0031	0.0083	0.0022	0.0052
0.0016	0.0048	0.0020	0.0034
Control	0.0025	0.0010	0.0018
	<0.0005	<0.0005	<0.0005
<i>H. azteca</i>			
0.010	0.116	0.0258	0.071
0.050	0.057	0.0133	0.035
0.025	0.030	0.0078	0.019
0.0125	0.016	0.0035	0.010
0.0062	0.0083	0.0025	0.0054
0.0031	0.0048	0.0018	0.0033
0.0016	0.0025	0.0007	0.0016
Control	<0.0005	<0.0005	<0.0005

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Cypermethrin LC50 H. azteca

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.071	19	19	100	1.907348E-04
.035	19	19	100	1.907348E-04
.019	19	19	100	1.907348E-04
.01	19	17	89.4737	3.643036E-02
.0054	19	7	36.8421	17.96417
.0033	19	5	26.3158	3.178405
.0016	19	0	0	1.907348E-04

THE BINOMIAL TEST SHOWS THAT .0016 AND .01 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 6.218789E-03

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	4.831695E-02	6.78832E-03	5.498047E-03
4.321039E-03			

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
8	.1181897	1
.7329166		

SLOPE = 4.397862
 95 PERCENT CONFIDENCE LIMITS = 2.885933 AND 5.909791

LC50 = 5.446146E-03
 95 PERCENT CONFIDENCE LIMITS = 4.469644E-03 AND 6.636238E-03

LC10 = 2.800998E-03
 95 PERCENT CONFIDENCE LIMITS = 1.849562E-03 AND 3.549468E-03

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Cypermethrin LC50 C. riparius 4th instar

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2.9	17	5	29.4118	7.173156
1.4	17	6	35.2941	16.61529
.74	17	6	35.2941	16.61529
.33	17	0	0	7.629394E-04
.2	17	1	5.8824	1.373291E-02

THE BINOMIAL TEST SHOWS THAT .33 AND +INFINITY 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 .9782638

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	.5118	1

GOODNESS OF FIT PROBABILITY
9.924722E-02

SLOPE = 1.100414
95 PERCENT CONFIDENCE LIMITS = .3131756 AND 1.887652

LC50 = 4.64571
95 PERCENT CONFIDENCE LIMITS = 1.997649 AND 298.2298

LC10 = .3257923
95 PERCENT CONFIDENCE LIMITS = 1.684319E-02 AND .6616366

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Cypermethrin LC50 C. riparis 1st instar

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.072	17	17	100	7.629394E-04
.037	17	17	100	7.629394E-04
.019	17	17	100	7.629394E-04
.01	17	12	70.58821	7.173156
.0052	17	6	35.2941	16.61529
.0034	17	0	0	7.629394E-04
.0018	20	2	10	2.012253E-02

THE BINOMIAL TEST SHOWS THAT .0034 AND .019 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 6.813822E-03

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	9.712188E-02	9.700744E-03	7.186031E-03
5.358179E-03			

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	.101594	1
7.845551E-02		

SLOPE = 3.586193
 95 PERCENT CONFIDENCE LIMITS = 2.443137 AND 4.729249

LC50 = 6.58577E-03
 95 PERCENT CONFIDENCE LIMITS = 5.26574E-03 AND 8.328458E-03

LC10 = 2.913872E-03
 95 PERCENT CONFIDENCE LIMITS = 1.873085E-03 AND 3.811549E-03
