

**Study Title**

Cypermethrin :  
Toxicity to *Chironomus riparius* and *Hyalella azteca*

**Authors**

[REDACTED]

**Data Requirement**

Supplemental

**Study Completed on**

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**Performing Laboratory**

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**Submitted by**

[REDACTED]

Study Number : [REDACTED]

Report Title : Cypermethrin : Toxicity to *Chironomus riparius* and *Hyaella azteca*

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Study Number : 95JH082

Report Title : Cypermethrin : Toxicity to *Chironomus riparius* and *Hyalella azteca*

#### GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

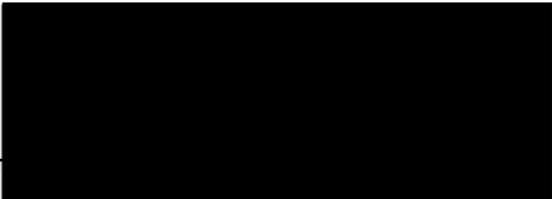
This study has been carried out in compliance with the Principles of Good Laboratory Practice laid down in the United Kingdom Department of Health Compliance Programme (1989). These Principles are consistent with the Organisation of Economic Co-operation and Development Principles of Good Laboratory Practice OCDE/GD(92)32.

This study is therefore considered to satisfy the requirement that it be conducted in accordance with 40 CFR Part 160.

This study is valid for the purposes for which it was conducted and this report is a true reflection of the raw data generated.

S  

Submitter 

Sponsor 

Study Number : 95JH082

Report Title : Cypermethrin : Toxicity to *Chironomus riparius* and *Hyaella azteca*.

#### QUALITY ASSURANCE STATEMENT

In accordance with [REDACTED] policy and procedures for Good Laboratory Practice, the conduct of this study has been inspected/audited by the Quality Assurance Section at [REDACTED].

| Date of Inspection | Inspection/Audit | Date of Inspection Report |
|--------------------|------------------|---------------------------|
| 07 Apr 1995        | Protocol         | 07 Apr 1995               |
| 19 Jun 1996        | Final Report     | 26 Jun 1996               |

In addition, the following facility and procedure inspections associated with this type of study have been carried out.

|             |                       |             |
|-------------|-----------------------|-------------|
| 01 Aug 1995 | Laboratory Facilities | 04 Apr 1996 |
|-------------|-----------------------|-------------|

So far as can be reasonably established, the methods described and results incorporated in this report accurately reflect the raw data produced during the study.

[REDACTED]

[REDACTED]

23 July 1996  
Date

Study Number : 95JH082

Report Title : Cypermethrin : Toxicity to *Chironomus riparius* and *Hyalella azteca*

#### AUTHENTICATION

I, the undersigned, hereby declare that this study was performed under my direction and that this report represents a true and accurate record of the results obtained.

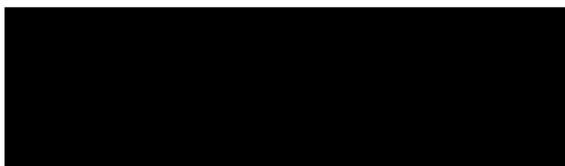


22nd July 1996  
Date



18/7/96  
Date

Authorised for Management by :



23 July 1996  
Date

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Authors : XXXXXXXXXX

#### SUMMARY

The toxicity of <sup>14</sup>C-cypermethrin to first and fourth instar *Chironomus riparius* and 7-14 day old *Hyalella azteca* was determined. Test organisms were exposed to a series of concentrations of the test chemical in water for 48 hours. Based on mean measured concentrations of <sup>14</sup>C-cypermethrin, 48 hour LC<sub>50</sub> values were 0.0069, >2.9 and 0.0053 µg l<sup>-1</sup>, for first and fourth instar *C. riparius* and 7-14 day old *H. azteca*, respectively.

1           **INTRODUCTION**

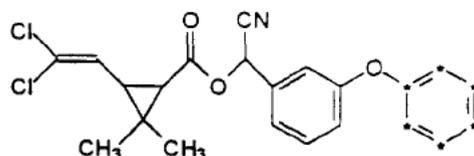
Cypermethrin is a broad spectrum pyrethroid insecticide used to control a wide variety of agricultural pests. In normal agricultural use, entry into non-target aquatic environments may occur through spray drift or run-off. The present study was designed to investigate the toxicity of the cypermethrin to first and fourth instar *Chironomus riparius* larvae and 7-14 day old *Hyalella azteca*, chosen as representative benthic invertebrates.

2           **MATERIALS AND METHODS**

2.1       **Test Chemical**

Radiolabelled cypermethrin was used in order to facilitate the chemical analysis of the low concentrations. <sup>14</sup>C-phenoxy-labelled cypermethrin (see Figure 1) was supplied by the [REDACTED], with a specific activity of 2.1 G Bq mmol<sup>-1</sup> (Ref 93-J28). The radiochemical purity of the stock solution was determined by thin layer chromatography (TLC, see Section 2.7) at >89%.

**Figure 1 : Molecular Structure of Cypermethrin**



\* position of radiolabel

IUPAC Name:

(*RS*)- $\alpha$ -cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CA Name:

cyano(3-phenoxyphenyl)methyl 3-(2,2 dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate

The water solubility of cypermethrin at pH7 is 4  $\mu\text{g l}^{-1}$  (Reference 1).

## 2.2 Test Organisms

### 2.2.1 *Chironomus riparius*

*Chironomus riparius* were obtained from cultures maintained at Jealotts Hill. Culture vessels were 5 litre plastic aquaria with a bottom layer of approximately 2 cm silver sand and 12 cm water (hardness approximately 170 mg l<sup>-1</sup>). The cultures were maintained at approximately 23°C, on a 16 hour : 8 hour light:dark cycle and fed ground tetra-min *ad libitum*. Under these conditions the life-cycle of *Chironomus riparius* is approximately 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 instars were counted out from established cultures. Example test organisms are shown in Appendix I, Figure 2.

### 2.2.2 *Hyalella azteca*

*Hyalella azteca* were obtained from laboratory cultures at [REDACTED]. Mixed age cultures were maintained in hard water (see Section 2.3) at 23°C on a 16 hour : 8 hour light:dark cycle. Culture vessels were 20 litre glass aquaria containing approximately 10 litres. Horsechestnut (*Aesculus hippocastanum*) leaves, dried then pre-conditioned by soaking in hard water, were added as a substrate and food.

The *H. azteca* were 7 to 14 days old at the start of the test (see Appendix I, Figure 3). Organisms <7 days old were sorted from the culture (those which pass through a 500 µm sieve but are retained on a 250 µm sieve) and transferred to a fresh culture vessel for a further 7 days.

## 2.3 Test Method

The test organisms were exposed to a range of concentrations of the test chemical for 48 hours in order to determine LC50 values. The dilution water used to prepare the test solutions was a hard water (166 mg l<sup>-1</sup> CaCO<sub>3</sub>), produced by mixing dechlorinated mains water (hardness approximately 300 mg l<sup>-1</sup>) with the same water which had been deionised using a reverse osmosis system.

The nominal test concentrations were :

*H. azteca* & 1st instar *C. riparius*: 0.1, 0.05, 0.025, 0.0125, 0.0062, 0.0031  
and 0.0016 µg l<sup>-1</sup>

4th instar *C. riparius* : 4, 2, 1, 0.5 and 0.250 µg l<sup>-1</sup>

Stock solutions of the <sup>14</sup>C-labelled cypermethrin in acetone were prepared for each test concentration. Addition of the acetone stocks to the test water gave the required nominal test concentrations in 0.01% acetone. Both untreated controls and solvent controls, containing acetone at 0.01%, were prepared.

Test vessels were 250 ml, 600 ml and 2 litre glass beakers for testing first instar *C. riparius*, *H. azteca* and fourth instar *C. riparius*, respectively, containing 220 ml, 400 ml and 1 litre of test solution, respectively. 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 maintained in a constant temperature room at  $23 \pm 2^\circ\text{C}$  under fluorescent lighting providing approximately 600 lux on a 16 hour: 8 hour light:dark cycle. The organisms were not fed during the test. The organisms were assessed for mortality (no visible movement) at 48 hours. The 1st instar *C. riparius* were assessed with the aid of a 2x magnifying lens.

## 2.4 Statistical Analysis of Biological Results

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

## 2.5 Analysis of Test Preparations

### 2.5.1 Dissolved Oxygen, pH and Temperature

At 0 and 48 hours, dissolved oxygen and pH were measured in each test vessel using YSI Model 57 and Radiometer PHM62 meters, respectively. Temperature was recorded throughout the test using a temperature probe connected to a data logger. Readings were taken automatically every 30 minutes.

### 2.5.2 Analysis of Test Chemical

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  $^{14}\text{C}$ -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. Prior to the test, recovery experiments were conducted using this extraction procedure. At measured aqueous concentrations over the range 0.01 to  $3.6 \mu\text{g l}^{-1}$ , recoveries in the hexane ranged from 83-114%.

At the two highest concentrations aliquots of the hexane extracts were analysed by TLC to confirm the extracted radioactivity was cypermethrin.

## 2.6 Liquid Scintillation Counting

LSC counts were carried out using an LKB 1217 Rackbeta 'Spectral' spectrophotometer. The scintillation cocktail used was 'Optiphase Safe' (LKB Sweden).

Each group of samples was preceded by two blank samples from which a mean was calculated. The mean background count was automatically subtracted from subsequent count rates in the batch to provide net disintegrations per second (DPS) values.

## 2.7 Thin Layer Chromatography

Thin layer chromatography was used to determine the purity of the  $^{14}\text{C}$ -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).

In addition, TLC was used to characterise the radioactivity extracted from water at 48 hours at the two highest concentrations using solvent system 2.

## 3 RESULTS AND DISCUSSION

### 3.1 Dissolved Oxygen, pH and Temperature

Dissolved oxygen levels remained  $\geq 7.8 \text{ mg l}^{-1}$  and pH was in the range 7.8 - 8.3. The mean temperature of the constant temperature room was  $22.6^\circ\text{C}$  during the period of the test, range  $22.0\text{-}23.4^\circ\text{C}$ .

### 3.2 Concentration of Test Chemical

The measured concentrations of  $^{14}\text{C}$ -cypermethrin are shown in Table 1. Measured concentrations were in the range 91-156% of nominal at 0 hours. The measured concentrations being close to nominal particularly at the higher concentrations of the 4th instar *C. riparius* confirmed the stability of the cypermethrin in the acetone stock solutions. Measured concentrations had fallen after 48 hours in all the test solutions, to be in the range 42-51% of nominal for the test solutions containing 4th instar *C. riparius* and 28-65% and 26-58% for 1st instar *C. riparius* and *H. azteca* test solutions, respectively.

### 3.3 Toxicity Data

The 48 hour  $\text{LC}_{50}$  values are shown in Table 2 and the full biological results are contained in Appendix II. The 48 hour  $\text{LC}_{50}$  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  $\text{LC}_{50}$  was  $4.6 \mu\text{g l}^{-1}$ , although this value has very wide confidence limits and is outside the range of concentrations tested. Thus the  $\text{LC}_{50}$  is best quoted as  $>2.9 \mu\text{g l}^{-1}$ , the highest mean measured concentration tested (to 2 significant figures).

**Table 1 : Measured Concentrations of Cypermethrin in Test Solutions**

| Nominal Concentration ( $\mu\text{g l}^{-1}$ ) | Measured Concentration ( $\mu\text{g l}^{-1}$ ) <sup>ab</sup> |             |              |
|--|---|-------------|--------------|
|  | 0 hr  | 48 hr       | Mean         |
| <u><i>C. riparius</i> 4th instar</u>           |   |             |              |
| 4.00   | 3.74 (93)   | 2.04 (51)   | 2.89 (72)    |
| 2.00   | 1.95 (97)   | 0.886 (44)  | 1.42 (71)    |
| 1.00   | 1.01 (101)  | 0.465 (46)  | 0.736 (74)   |
| 0.50   | 0.454 (91)  | 0.211 (42)  | 0.332 (66)   |
| 0.25   | 0.279 (112)   | 0.128 (51)  | 0.204 (82)   |
| Control  | <0.0005 <sup>d</sup>  | <0.0005     | <0.0005      |
| <u><i>C. riparius</i> 1st instar</u>           |   |             |              |
| 0.100  | 0.116 <sup>b</sup> (116)                                      | 0.0283 (28) | 0.072 (72)   |
| 0.050  | 0.057 (114)   | 0.0172 (34) | 0.037 (74)   |
| 0.025  | 0.030 (119)   | 0.0078 (31) | 0.019 (76)   |
| 0.0125   | 0.016 (130)   | 0.0037 (30) | 0.010 (80)   |
| 0.0062   | 0.0083(129)   | 0.0022 (35) | 0.0052 (84)  |
| 0.0031   | 0.0048(155)   | 0.0020 (65) | 0.0034(110)  |
| 0.0016   | 0.0025(156)   | 0.0010 (62) | 0.0018(112)  |
| Control  | <0.0005   | <0.0005     | <0.0005      |
| <u><i>H. azteca</i></u>                        |   |             |              |
| 0.100  | 0.116 <sup>b</sup> (116)                                      | 0.0258 (26) | 0.071 (71)   |
| 0.050  | 0.057 (114)   | 0.0133 (27) | 0.035 (70)   |
| 0.025  | 0.030 (119)   | 0.0078 (31) | 0.019 (76)   |
| 0.0125   | 0.016 (130)   | 0.0035 (28) | 0.010 (80)   |
| 0.0062   | 0.0083 (129)  | 0.0025 (40) | 0.0054 (87)  |
| 0.0031   | 0.0048 (155)  | 0.0018 (58) | 0.0033 (106) |
| 0.0016   | 0.0025 (156)  | 0.0007 (44) | 0.0016 (100) |
| Control  | <0.0005   | <0.0005     | <0.0005      |

<sup>a</sup> assuming all <sup>14</sup>C measured by LSC is <sup>14</sup>C-cypermethrin

<sup>b</sup> figures in brackets are measured concentrations as % nominal

<sup>c</sup> 1st instar *Chironomus* and *Hyalella* test concentrations were from the same bulk preparation and so have the same measured concentration at 0 hours.

<sup>d</sup> approximate limit of determination for the method used

**Table 2 : Toxicity of Cypermethrin to *Chironomus* and *Hyalella***

| Test Organism                              | 48 hour LC <sub>50</sub> Values<br>( $\mu\text{g l}^{-1}$ ) | 95% Confidence Limits |
|--|---|-----------------------|
| <i>Chironomus riparius</i><br>(1st instar) | 0.0069  | 0.0056-0.0085         |
| <i>Chironomus riparius</i><br>(4th instar) | 4.6   | 2-600                 |
| <i>Hyalella azteca</i><br>(7-14 days old)  | 0.0053  | 0.0043-0.0065         |

4 **STUDY PERSONNEL**

This study was carried out by [REDACTED] and [REDACTED] in the Ecological Risk Assessment Section at [REDACTED]. The statistical analyses were carried out by [REDACTED] of the [REDACTED].

5 **STUDY DATES**

The study was carried out between March and May 1995.

6 **RAW DATA**

The raw data for this study are contained in a study file bearing the study number 95JH082. The raw data and a copy of this report are stored in [REDACTED].

7 **REFERENCES**

1. Tomlin C ed (1994). The Pesticide Manual (Tenth Edition). BCPC Publications.

Filename : RC0002.doc  
Location : E:\GROUP\WP\RC  
Reference : MJH/JD  
WINo : 20874  
Date : 24 July 1996

Appendix I : Test Organisms

Figure 2 : *Chironomus riparius*, 1st and 4th instar

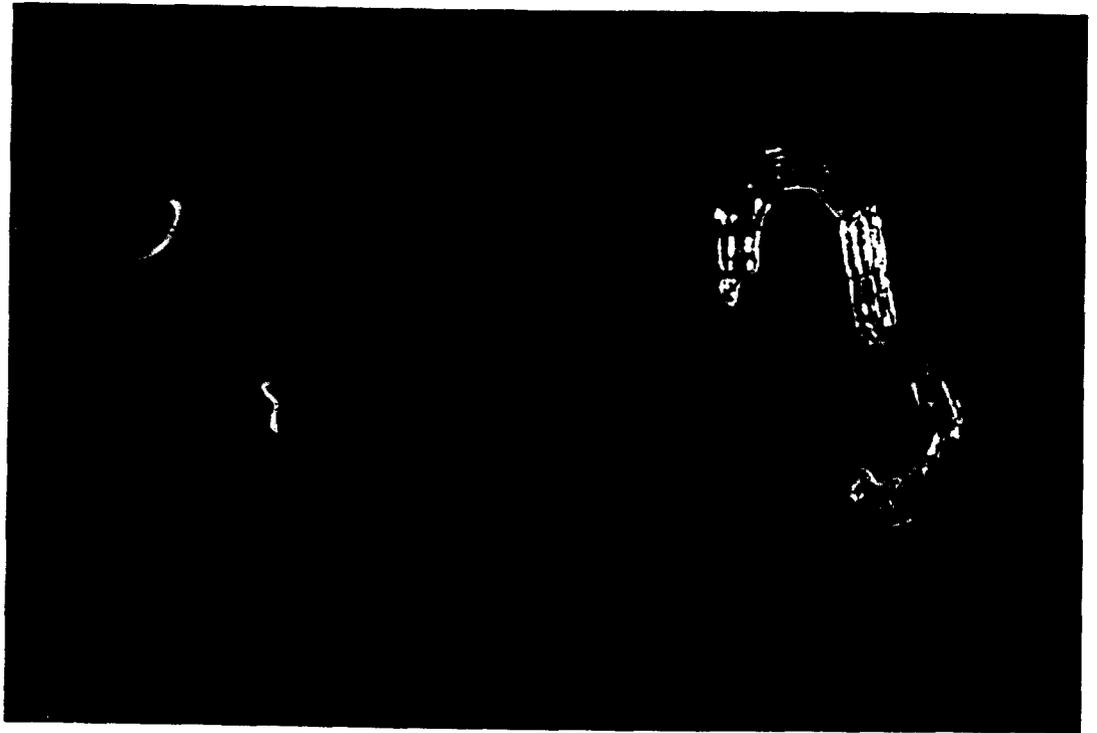


Figure 3 : *Hyalella azteca*, 7-14 days old



**Appendix II : Biological Results**

**Table 3 : Toxicity of Cypermethrin to First Instar *Chironomus riparius***

| Mean Measured Concentration ( $\mu\text{g l}^{-1}$ ) | Number Dead at 48 hr (out of 20) |
|--|----------------------------------|
| 0.072  | 20                               |
| 0.037  | 20                               |
| 0.019  | 20                               |
| 0.010  | 15                               |
| 0.0052   | 9                                |
| 0.0034   | 3                                |
| 0.0018   | 2                                |
| Solvent control                                      | 3                                |
| Untreated control                                    | 2                                |

**Table 4 : Toxicity of Cypermethrin to Fourth Instar *Chironomus riparius***

| Mean Measured Concentration ( $\mu\text{g l}^{-1}$ ) | Number Dead at 48 hr (out of 20) |
|--|----------------------------------|
| 2.9  | 8                                |
| 1.4  | 9                                |
| 0.74   | 9                                |
| 0.33   | 3                                |
| 0.20   | 4                                |
| Solvent control                                      | 3                                |
| Untreated control                                    | 3                                |

**Table 5 : Toxicity of Cypermethrin to 7-14 day old *Hyaella azteca***

| Mean Measured Concentration ( $\mu\text{g l}^{-1}$ ) | Number Dead at 48 hr (out of 20) |
|--|----------------------------------|
| 0.071  | 20                               |
| 0.035  | 20                               |
| 0.019  | 20                               |
| 0.010  | 18                               |
| 0.0054   | 8                                |
| 0.0033   | 6                                |
| 0.0016   | 1                                |
| Solvent control                                      | 1                                |
| Untreated control                                    | 0                                |