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AMENDMENT TO DER BY T. JOHNSTON, 4/13/82

- 1. CHEMICAL: Cypermethrin
- 2. FORMULATION: 93-94% active ingredient (technical grade),  
14-C labeled compound
- 3. STUDY/ACTION TYPE: Daphnia 21-day chronic toxicity  
Daphnia magna (first instar)
- 4. CITATION: Cypermethrin: 21-day Daphnia magna life cycle study. Submitted by ICI Americas for Phase 4 List B review. MRID 92027-025 (Summary Report) and MRID 00089047 (Study Report).

*Edwards et al.*

- 5. REVIEWED BY: Ann Stavola  
Aquatic Biologist  
EEB/EFED
- 6. APPROVED BY: Charles Lewis  
Acting Section Head  
EEB/EFED

Signature: *Ann Stavola*  
Date: *1/8/91*

Signature: *Charles Lewis*  
Date: *1/10/91*

7. CONCLUSIONS: The original DER was re-evaluated based upon the summary report and the original study in order to clarify some aspects of the original DER. In addition, the original DER did not indicate that the author's results were verified by reanalyzing the data statistically. The classification of the study is being changed as it does not meet current EPA guideline requirements for a Daphnia life cycle study. The reasons are:

There was only one test chamber per test concentration; therefore in order to conduct statistical analyses the results from two separate tests run a month apart were combined. This is not acceptable because the conditions during the two tests were different; therefore they are not true replicates. The number of adults per chamber were culled on test day 9; this is not an acceptable procedure for an invertebrate life cycle study. The design of the study (30 reproducing adults in one chamber) does not provide for accurate observations of reproduction per female.

Therefore, the study is determined to be classified as not scientifically sound, and it does not meet our guideline requirements.

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8. RECOMMENDATIONS: A new Daphnia 21-day life cycle study, preferably using the renewal procedure, is required. Endpoints are survival, production of young and dry weight of first generation daphnids. The registrant should refer to ASTM standard guide E-1193 for guidance.

DATA EVALUATION

1. CHEMICAL: Cypermethrin
2. FORMULATION: 93-94 % active ingredient (Technical grade, cis:  
trans ratio = 55:45, <sup>14</sup>C-labeled compound)
3. CITATION: Edwards, P.J., M.J. Hamer, J.M. Bull, and S. M. Brown  
(1981) cypermethrin: 21-day Daphnia magna life cycle  
study. Unpublished report by the Plant Protection  
Division, submitted 12/28/81 by ICI Americas Inc.,  
Wilmington, Delaware.

EPA Accession No. 070562

MRID 00089047

REVIEWED BY: Thomas B. Johnston  
Biologist, EEB/HED

REVIEW DATE: April 13, 1982

TEST TYPE: 21-day life cycle

REPORTED RESULTS: The reported 21-day MATC of cypermethrin for  
Daphnia magna is between 9 and 20 pptr.  
It is concluded that the chronic effects of  
cypermethrin on D. magna are of little  
importance compared with acute effects.

REVIEWER'S CONCLUSIONS: This study is scientifically sound, and  
fulfills USEPA guideline requirements for  
a chronic life-cycle toxicity test using  
an aquatic invertebrate. With a 21-day  
MATC of between 9 and 20 pptr, cypermethrin  
is very highly toxic to Daphnia magna.  
The reviewer concurs with the experimenter's  
conclusion that reproductive impairment  
does not occur at significantly lower con-  
centrations than acute toxicity.

## MATERIALS METHODS

Methods used generally followed USEPA guidelines. Tests were run at 17°C. Daphnids were fed algae twice daily. Eighty 1<sup>st</sup> instar Daphnia were introduced into each chamber. On exposure day 9, survivors were counted, and 30 parthenogenic females were selected and returned to the test Chambers. On exposure days 14,17, and 21 (Test 1) and 13,17,21, and 22 (Test 2) all parthenogenic females were removed and counted. Young were also collected and counted at this time.

At the end of each test, the body lengths of 10 randomly selected females from each treatment were measured. In addition, 20 young Daphnia from each treatment were held for 13 days in static dechlorinated water to determine if the fertility of daphnids born in water containing cypermethrin was affected. Survival of initial young and their offspring were both assessed.

## RESULTS

Table 1 Effect of cypermethrin on number of Daphnia produced per parthenogenic female

Tests 1 and 2 (days 21) used as separate blocks in the analysis of variance.

Test Chambers	Mean Measured Concentration from Test 1 and Test 2 (ng/l)	OFFSPRING/FEMALE	
		Adjusted for Adult Mortality	Unadjusted (Based on 30 individuals)
A	0	51.0 A	49.7 A
B	5.4	53.5 A	53.6 A
C	11.5	45.5 AB	45.4 A
D	20.1	38.0 AB	38.0 A
E	42.1	32.0 BC	31.3 AB
F	81.3	16.5 C	12.7 B (I)
Standard Error (single plot)		7.3	9.0
Coefficient of variation		18.5%	23.3%
Diff. between Treatment means		SIG 18.7	SIG 23.0
LSD p=5%		29.4	36.1
LSD p=1%			

Table 1 (continued)

Test 1 (day 21) and Test 2 (day 22) used as separate blocks in the analysis of variance

Test Chambers	Mean Measured Concentration from Test 1 and Test 2 (ng/l)	OFFSPRING/FEMALE	
		Adjusted for Adult Mortality	Unadjusted (Based on 30 individuals)
A	0	54.0 A	52.6 A
B	5.4	55.5 A	55.6 A
C	11.5	52.0 A	52.3 AB
D	20.1	43.0 AB	40.6 AB
E	42.1	37.0 B	36.1 B
F	81.3	17.0 C	13.3 C (I)
Standard Error (single plot)		5.1	6.4
Coefficient of variation		11.9%	15.3%
Diff. between Treatment means		H.SIG	H.SIG
LSD p=5%		13.2	16.5
LSD p=1%		20.8	25.8

Treatment means with no letter in common are significantly different at the 5% probability level.

Table 2 Effect of cypermethrin on length of *Daphnia magna*

Test Chamber	TEST 1		TEST 2	
	Mean measured con'c	Length of parthenogenic	Mean measured con'c	Length of parthenogenic
A	0	38.7 A	0	39.4 A
B	4.7	38.6 AB	6.0	38.8 A
C	9.3	39.6 A	13.6	36.2 B
D	17.2	37.3 B	23.0	35.7 B
E	39.8	34.8 C	44.3	31.1 C
F	79.2	26.7 D	83.4	31.0 C
Standard Error (single plot)		1.5		1.3
Coefficient of variation		4.1%		3.7%
Diff. between Treatment means		H.SIG		H.SIG
LSD p=5%		1.3		1.2
LSD p=1%		1.8		1.6

Numbers quoted are micrometer eyepiece graticules (50 graticules = 4.7mm)

Test 1 body length measured on Day 21

Test 2 body length measured on Day 22

Treatment means with no letter in common are significantly different at the 5% probability level.

Table 3 Cypermethrin dose to give a 50% reduction in number of off-spring per parthenogenic female (EC<sub>50</sub>), based on mean measured concentrations

(i) Not adjusted for Adult Mortality

Test No/time	EC <sub>50</sub> (ng/l)	95% confidence limits
1/21 day	48.4	37.8 - 58.9
2/21 day	62.9	36.7 - 89.1
2/22 day	70.4	59.4 - 81.3

(ii) Adjusted for Adult Mortality

Test No/time	EC <sub>50</sub>	95% confidence limits
1/21 day	52.8	41.1 - 64.6
2/21 day	66.0	38.3 - 93.8
2/22 day	71.5	62.5 - 80.5



Table 4a      Toxicity of cypermethrin to adult *Daphnia magna* after 9 days exposure

Test Chamber	Test 1		Test 2	
	Mean measured con'c (ng/l)	% mortality	Mean measured con'c (ng/l)	% mortality
A	0	3	0	3
B	4.7	6	6.0	3
C	9.3	0	13.6	10
D	17.2	4	23.0	6
E	39.8	15	44.3	6
F	79.2	51	83.4	38

% mortality based on 80 1st instar *Daphnia magna*, initially introduced to test chambers

Table 4b      Toxicity of cypermethrin to adult *Daphnia magna* between 9 and 21 days exposure

Test Chamber	Test 1		Test 2	
	Mean measured con'c (ng/l)	% mortality	Mean measured con'c (ng/l)	% mortality
A	0	7	0	3
B	4.7	0	6.0	0
C	9.3	0	13.6	0
D	17.2	0	23.0	0
E	39.8	0	44.3	3
F	79.2	30	83.4	47

% mortality based on 30, 9 day old *Daphnia magna*, introduced into test chambers

Table 5a Effect of cypermethrin on survival of introduced Daphnia and numbers of off-spring in Test 1

Test 1 - Started 12th August, 1980

Test chamber	Mean Measured con'c (ng/l)	Day 0 1st instar introduced	Day 9 Total surviving selected	Day 14 surviving	Day 17		Day 21		TOTAL Young		
					Young surviving	Young surviving	Surviving	Young			
A	0	80	78	30	30	401	30	586	28	704	1691
B	4.7	80	75	30	30	401	30	584	30	799	1784
C	9.3	80	80	30	30	435	30	597	30	740	1772
D	17.2	80	77	30	30	361	30	416	30	474	1251
E	39.8	80	68	30 (1)	30 (1)	337	30 (2)	250	30 (3)	542	1129
F	79.2	80	39	30	28	0	28	17	21	65	82

(1) growth retarded, includes males and females.

(2) 12 females, 16 males.

(3) 6 females, 15 males.

Table 5b - Effect of cypermethrin on survival of introduced Daphnia and number of off-spring in Test 2

Test 2 - Started 3rd September 1980

Test Chamber	Mean Measured con'c (ng/l)	Day 0	Day 9		Day 13		Day 17		Day 21	
		1st instar introduced	Total Surviving	Females Selected	Females Surviving	Young	Females Surviving	Young	Females Surviving	Young
A	0	80	78	30	30	374	30	458	29	462
B	6.0	80	78	30	30	392	30	526	30	512
C	13.6	80	72	30	30	217	30	357	30	379
D	23.0	80	75	30	30	342	30	403	30	294
E	44.3	80	75	30	29	132	29	308	29	314
F	83.4	80	50	30(1)	28(2)	8	28(2)	178	14(3)	241

Day 22		Final TOTAL Young
Females Surviving	Young	
29	174	1468
30	121	1551
30	412	1365
30	156	1185
29	283	1037
19(4)	28	455

(1) Growth retarded, includes males and females

(2) 23 females, 5 males.

(3) 11 females, 3 males.

(4) Poor condition, not possible to measure length or determine sex in all but 6 (3 females and 3 males.)

CONCLUSIONS:

Validation Category: Core

Category Rationale: N/A

Category Repairability: N/A