

US EPA ARCHIVE DOCUMENT

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

APR 7 1992

MEMORANDUM

SUBJECT: Review of Freshwater Invertebrate Life-Cycle Chronic Toxicity Test

FROM: Douglas Urban, Acting Chief
Ecological Effects Branch (H-7507C)
Environmental Fate And Effects Division

TO: Joanne Edwards, PM
Accelerated Reregistration Branch (H-7508W)
Special Review And Reregistration Division

The Ecological Effects Branch (EEB) has reviewed the attached freshwater invertebrate chronic toxicity tests in which Daphnia magna were exposed to Cythion (94% technical) in a 21-day flow through study (72-4B). This study was listed as a requirement in the Registration Standard of February 1988. The study was scientifically sound and categorized as CORE. The 21-day EC50 of Cythion for D. magna was 0.52 µg/L. The MATC was based on the most sensitive biological parameter, daphnid reproduction (young produced/adult reproductive day). The MATC was > 0.06 µg/L and < 0.10 µg/L mean measured concentration (geometric mean = 0.077 µg/L). On the basis of adult daphnid length, a NOEC of 0.10 µg/L mean measured concentration was determined.

The following cited study satisfies guideline requirements as prescribed in the Registration Standard of 1988.

Blakemore, G. and D. Burgess. 1990. Chronic toxicity of Cythion to Daphnia magna under flow-through test conditions. Laboratory Report No. 37399. Prepared by Analytical Biochemistry Laboratories, Inc., Columbia, MO. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 417184-01.

If you have any questions regarding this review, please call Tom A. Bailey at 703-305-6666. ①

CONCURRENCES

SYMBOL	H-7507C	H-7507C	H-7507C				
SURNAME	T. Bailey	Cramer	D. Urban				
DATE	4-7-92	4/7/92	4/8/92				



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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

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2



9. BACKGROUND:10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

- A. Test Animals: Daphnia magna (<24 hours old) were obtained from in-house cultures. The primary culture was obtained from the Columbia National Fisheries Research Laboratory in Columbia, MO. The cultures were housed in a temperature controlled area ($20^{\circ}\pm 2^{\circ}\text{C}$) on a 16-hour daylight photoperiod with 30 minute dawn/dusk simulations. The light intensity was maintained at 40-80 ft-candles.

Adult daphnids were fed a suspension of algae (Selenastrum capricornutum and Ankistrodesmus falcatus) supplemented with a trout chow and yeast suspension.

- B. Test System: The proportional diluter delivered 3.6 mL/chamber/minute to each of four 1-liter test vessels per concentration (or 5.2 volume replacements per day). Flow splitting chambers were used to mix and divide each test solution. To minimize turbulence, the solutions were delivered to the test vessels using 14-gauge hypodermic needles. The system was calibrated before use and Cythion was allowed to run through the system for three days.

The test vessels were glass beakers with notched drains covered by 50-mesh stainless steel screens. The test chambers were immersed in a temperature-controlled water bath set to $20^{\circ}\pm 1^{\circ}\text{C}$. The photoperiod was the same as in culturing with a light intensity of 44-52 ft-candles.

The characteristics of the hard dilution water are given in Table 1 (attached).

The test substance was dissolved in dimethylformamide (DMF). The resulting stock solution was delivered to the diluter using a syringe dispenser.

- C. Dosage: Twenty-one-day, flow-through, life-cycle chronic toxicity test. Based on a preliminary test, five nominal concentrations (0.06, 0.12, 0.25, 0.50, and 1.0 $\mu\text{g/L}$), a dilution water control and a solvent control (0.05 mL DMF/L) were selected for the test.

The nominal concentrations were adjusted to reflect 100% of active ingredient.

- D. **Design:** Four chambers were used for each concentration with ten randomly-placed daphnids per chamber. All chambers were observed at daily for mortality, abnormal effects and the release of the first brood. Young were counted every Monday, Wednesday, and Friday by removing the adult with a smooth glass pipet and pouring the test solution through a 50-mesh stainless steel screen. The collected young were placed in sample jars, counted, and discarded. The solution was collected and replaced along with the adult daphnids back into the chamber. The test chambers were cleaned on each counting day. At test termination, the daphnids were individually measured.

The daphnids were fed Selenastrum capricornutum 4 times daily and Ankistrodesmus falcatus 2 times daily providing at least 6×10^6 cells/L. They were also supplemented twice daily with 1 mL of trout chow/yeast suspension (2 mg solids/mL).

The temperature, dissolved oxygen (D.O.), and pH were measured in the dilution water control, low, middle, and high concentration on days 0, 4, 7, 14, and 21. The temperature of the water bath was measured continuously with a data logger. The above parameters and conductivity, hardness, and alkalinity of the dilution water were measured daily.

Cythion concentrations were measured by gas-liquid chromatography from samples taken on days 0, 4, 7, 14, and 21.

- E. **Statistics:** Daphnid survival, growth (length), and reproduction (time to first brood and young/adult reproduction days) were analyzed statistically. Survival was analyzed using a frequency analysis to compare the concentrations to the control. Daphnid length and reproduction were analyzed using one-way analysis of variance (ANOVA) with subsequent means testing. The 21-day EC_{50} was determined using a program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.060, 0.10, 0.25, 0.46, and 0.94 $\mu\text{g/L}$ and averaged $94 \pm 7.0\%$ of nominal. Measured concentrations were fairly consistent between sampling days (Table 2, attached).

Daphnid survival in the 0.46 and 0.94 $\mu\text{g/L}$ concentrations were significantly lower than that of the pooled controls after 21 days (Table 4, attached). The 21-day EC_{50} was 0.52 $\mu\text{g/L}$. Adult daphnid lengths at 0.25 and 0.46 $\mu\text{g/L}$ were significantly lower than that of the pooled controls (Table 4, attached). "While a statistical difference was identified for the mean measured concentration of 0.10 $\mu\text{g/L}$, it was not considered to be a deleterious effect. It has been our past experience that a test level with a mean so close to that of the pooled controls mean, has not been considered biologically effected."

Time to first brood (9 and 10 days) in the two highest concentrations, 0.46 and 0.94 $\mu\text{g/L}$, was significantly increased (Table 5, attached). The time to brood in all other concentrations was 7 days. The number of young per adult reproductive day was significantly lower than the pooled controls in all concentrations except for the lowest, 0.060 $\mu\text{g/L}$. All young produced during the study appeared normal.

Based on the analysis of survival, growth, and reproduction, the maximum acceptable toxicant concentration (MATC) limits were estimated to be 0.06 and 0.10 $\mu\text{g/L}$ (mean measured concentration) resulting in a geometric mean MATC of 0.077 $\mu\text{g/L}$.

The pH of the test solutions ranged from 8.1 to 8.5. Dissolved oxygen ranged from 6.8 to 8.8 mg/L or 80 to 104% of saturation at 21°C. The temperature of the test solutions were 21°-22°C during the study.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The author presented no conclusions other than those previously mentioned.

Quality Assurance and GLP Compliance Statements were included in the report indicating adherence to USEPA GLP Regulations.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by ASTM (1985), but deviated as follows:

The conductivity, hardness, and alkalinity of the dilution water were measured daily. ASTM (1985) states that these parameters must be measured on the control, low, medium, and high concentration test solutions weekly.

Treatments must be randomly assigned to the test chambers. The report does not mention if the treatments were randomly assigned.

- B. **Statistical Analysis:** The reviewer used one-way analysis of variance (Toxstat Version 3.3) to analyze the survival and reproduction (average number of young produced per adult reproductive day) of daphnids after 21 days. The negative control and solvent control were pooled prior to the analysis. The survival data were analyzed as proportional survival and arcsine square root transformed. The no-observed effect concentration (NOEC) for survival and reproduction were 0.25 and 0.06 $\mu\text{g/L}$, respectively (see attached printouts 1-4). The 21-day EC_{50} was determined using EPA's Toxanal program. The results were the same as the authors' (see attached printout 5). Adult daphnid length was analyzed using Crunch Version 3 and two-way analysis of variance. The NOEC was 0.10 $\mu\text{g/L}$ mean measured concentration (see printout 6).
- C. **Discussion/Results:** A copy of the results of continuous temperature monitoring (page 67) indicated that the temperature range of the water bath during the test was approximately 19.5° to 22.5°C. Since the temperature of the test solutions were measured regularly, the reviewer accepts the authors' values.

This study is scientifically sound and meets the guideline requirements for a chronic, flow-through toxicity test for the freshwater invertebrate, Daphnia magna. The 21-day EC_{50} was 0.52 $\mu\text{g/L}$. The MATC, based on the most sensitive biological parameter, daphnid reproduction, was $>0.06 \mu\text{g/L}$ and $<0.10 \mu\text{g/L}$ (geometric mean = 0.077 $\mu\text{g/L}$).

- D. **Adequacy of the Study:**
- (1) Classification: Core.
 - (2) Rationale: N/A.
 - (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 06-12-91.

REFERENCES: ASTM. 1985. Proposed Standard Practice for Conducting Daphnia magna Chronic Toxicity Tests in a Flow-Through System. Draft No. 4.

RIN 1244-00

Malathion EFED DTR

Page is not included in this copy.

Pages 9 through 12 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
- FIFRA registration data.
- The document is a duplicate of page(s) .
- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

417184-01 CYTHION, DAPHNIA MAGNA, 21-D REPRODUCTION
 File: A:41718401.DM1 Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN = 12.0750 CALCULATED t VALUE = 0.0000
 GRP2 (BLANK CRTL) MEAN = 12.0750 DEGREES OF FREEDOM = 6
 DIFFERENCE IN MEANS = 0.0000

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.0

TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.0

Shapiro Wilks test for normality

D = 2.877
 W = 0.958
 Critical W (P = 0.05) (n = 24) = 0.916
 Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

Bartlett's test for homogeneity of variance

Calculated B statistic = 3.03
 Table Chi-square value = 13.28 (alpha = 0.01)
 Table Chi-square value = 9.49 (alpha = 0.05)
 Average df used in calculation ==> df (avg n - 1) = 3.80
 Used for Chi-square table value ==> df (#groups-1) = 4

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	130.749	32.687	215.832
Within (Error)	19	2.878	0.151	
Total	23	133.626		

Critical F value = 2.90 (0.05,4,19)
 Since F > Critical F REJECT Ho:All groups equal

13

Printout 2

417184-01 CYTHION, DAPHNIA MAGNA, 21-D REPRODUCTION
File: A:41718401.DM1 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	12.075	12.075		
2	0.06	11.775	11.775	1.259	
3	0.12	10.125	10.125	8.183	*
4	0.25	7.975	7.975	17.204	*
5	0.5	5.900	5.900	25.911	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=19,4)

417184-01 CYTHION, DAPHNIA MAGNA, 21-D REPRODUCTION
File: A:41718401.DM1 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	0.06	4	0.580	4.8	0.300
3	0.12	4	0.580	4.8	1.950
4	0.25	4	0.580	4.8	4.100
5	0.5	4	0.580	4.8	6.175

Printout 3

417184-01, DAPHNIA MAGNA, CYTHION, 21-D SURVIVAL
 File: A:41718401.DM2 Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.340	4.840	7.640	4.840	1.340
OBSERVED	0	5	14	1	0

Calculated Chi-Square goodness of fit test statistic = 11.0264
 Table Chi-Square value (alpha = 0.01) = 13.277
 Data PASS normality test. Continue analysis.

Bartlett's test for homogeneity of variance

Calculated B statistic = 2.21
 Table Chi-square value = 13.28 (alpha = 0.01)
 Table Chi-square value = 9.49 (alpha = 0.05)
 Average df used in calculation ==> df (avg n - 1) = 3.00
 Used for Chi-square table value ==> df (#groups-1) = 4

Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	1.3713	CALCULATED t VALUE =	0.0000
GRP2 (BLANK CTRL) MEAN =	1.3713	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0000		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.0

TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.0

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	0.313	0.104	10.950
Within (Error)	16	0.153	0.010	
Total	19	0.466		

Critical F value = 3.24 (0.05,3,16)
 Since F > Critical F REJECT Ho:All groups equal

15

Printout 4

417184-01, DAPHNIA MAGNA, CYTHION, 21-D SURVIVAL

File: A:41718401.DM2

Transform: ARC SINE(SQUARE ROOT(Y))

BONFERRONI T-TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.371	0.975		
2	0.12	1.371	0.975	-0.000	
3	0.25	1.371	0.975	-0.000	
4	0.5	1.058	0.750	5.232	*

Bonferroni T table value = 2.33 (1 Tailed Value, P=0.05, df=16,3)

417184-01, DAPHNIA MAGNA, CYTHION, 21-D SURVIVAL

File: A:41718401.DM2

Transform: ARC SINE(SQUARE ROOT(Y))

BONFERRONI T-TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	0.12	4	0.071	7.3	0.000
3	0.25	4	0.071	7.3	0.000
4	0.5	4	0.071	7.3	0.225

417184-01, DAPHNIA MAGNA, CYTHION, 21-D SURVIVAL

File: A:41718401.DM2

Transform: ARC SINE(SQUARE ROOT(Y))

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT -

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	GRPS 1&2 POOLED	1.371				
2	0.12	1.371	26.00	13.00	4	
3	0.25	1.371	26.00	13.00	4	
4	0.5	1.058	11.00	13.00	4	*

Critical values use k = 3, are 1 tailed, and alpha = 0.05

16

Printout 5

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY. THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

RIFICI CYTHION DAPHNIA 6-11-91

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.94	40	40	100	0
.46	40	10	25	0
.25	40	1	2.5	0
.1	40	1	2.5	0
.06	40	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .5559102

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	2.795017E-02	.5225523	.474776 .5784959

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	22.10883	108.3215	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 5.282897
95 PERCENT CONFIDENCE LIMITS = -19.5573 AND 30.12309

LC50 = .5195512
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .2986964
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Printout 6

Fmax for testing homogeneity of between subjects variances: 636.67
 Number of variances= 24 df per variance= 8.

Analysis of Variance		Dependent variable: LENGHT			
Source	df	SS (H)	MSS	F	P
Between Subjects	225	10.6356			
C (CONC)	5	4.5583	0.9117	35.245	0.0000
R (REP)	3	0.1238	0.0413	1.595	0.1900
CR	15	0.7284	0.0486	1.877	0.0269
Subj w Groups	202	5.2251	0.0259		

Analysis of Variance File: CYTHION Date: 06-12-1991

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	4.328	6	3.860
2	4.254		
3	4.264		
4	4.260		
5	4.171		

Comparison	Bon- ferroni
1 > 2	
1 > 3	
1 > 4	
1 > 5	0.0004
1 > 6	0.0000
2 < 3	
2 < 4	
2 > 5	
2 > 6	0.0000
3 > 4	
3 > 5	
3 > 6	0.0000
4 > 5	
4 > 6	0.0000
5 > 6	0.0000

1 = negative control

2 = solvent control

3 = 0.06 µg/L

4 = 0.10 µg/L

5 = 0.25 µg/L

6 = 0.46 µg/L

mean measured concentrations

19

haughnessey # 057721

Chemical Name Melathion

Chemical Class _____

Page 1 of 1

Study/Species/Lab/
RID # _____

Chemical % a.i. _____
Results _____

Reviewer/ Validation
Date _____ Status _____

Chronic Fish

Concentrations Tested (ppm) - _____

Species:

MATC - > _____ < _____ ppm.

Sub:

Effected Parameters - _____

ID #

Control Mortality (%) - _____ Solvent Control Mortality (%) - _____

Comments:

Organic Invertebrate

94.0%

Concentrations Tested (ppm) - ^{*b} 0.060, 0.10^{0.10}, 0.25, 0.46, 0.94

Species: Daphnia magna

MATC - > 0.04 < 0.10 ppm ^{*b}.

Effected Parameters - Average number of young/female/Reproductive day ^①

~~Physical~~ Physical Biochemistry
LABORATORIES

Survival, growth

UC Core
12/1/91

4117184-01

Control Mortality (%) - 2.5 Solvent Control Mortality (%) - 2.5

Comments: