

MRID No. 40361808

DATA EVALUATION RECORD

1. CHEMICAL: Fenoxycarb
Shaughnessey Number: 125301
2. TEST MATERIAL: ^{14}C -Fenoxycarb; Ro 13-5223/024; 32.03 uCi/mg; 95 uCi; 99% radiopurity; a colorless crystal.
3. STUDY TYPE: Freshwater invertebrate chronic test.
Species Tested: Cladoceran (Daphnia magna)
4. CITATION: Forbis, A.D. 1987. Chronic Toxicity of ^{14}C -Fenoxycarb to Daphnia magna under Flow-Through Test Conditions. ABC Report No. 35568. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. Submitted by Maag Agrochemicals, Vero Beach, Florida. MRID No. 40361808.
5. REVIEWED BY:

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Kimberly D. Rhodes*
Date: *May 8, 1989*
6. APPROVED BY:

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*
Date: *May 8, 1989*

for Henry T. Craven
Supervisor, EEB/HED
USEPA
Henry T. Craven
11/15/89

Signature: *John Noles*
Date: *11/17/89*
7. CONCLUSIONS: This study appears to be scientifically sound and fulfills the guideline requirements for a freshwater invertebrate life-cycle flow-through test. The MATC of ^{14}C -Fenoxycarb for Daphnia magna was $> 1.6 \text{ ng a.i./L} < 2.3 \text{ ng a.i./L}$ based on mean measured concentrations.
8. RECOMMENDATIONS: N/A

9. BACKGROUND:10. DISCUSSION OF INDIVIDUAL TESTS: N/A11. MATERIALS AND METHODS:

A. Test Animals: Daphnia magna used to initiate the life-cycle test were obtained from the culture unit at the testing facility. All daphnids were cultured and tested in a temperature controlled area at $20 \pm 2^{\circ}\text{C}$. The lighting was 50-70 footcandles on a 16-hour daylight photoperiod, with 30-minute dawn and dusk transition periods. During the holding period, the daphnids were fed a suspension of algae (Selenastrum capricornutum) supplemented with a Tetramin, cereal leaves and yeast suspension. Only first-instar daphnids (< 24-hours old) were selected for testing.

B. Test System: The test was conducted in a half-liter proportional diluter system described by Mount and Brungs (1967), utilizing a Hamilton Micro Lab 420 syringe dispenser. Flow-splitting chambers were utilized to thoroughly mix and divide each ^{14}C -Fenoxycarb concentration for delivery to the test chambers. One-liter glass beakers, with notched drains which were covered with 50-mesh stainless steel screen to prevent escape of the daphnids, were used as the test chambers. The diluter delivered five concentrations of ^{14}C -Fenoxycarb, a dilution water control and a solvent control to four-replicate one-liter test chambers. The diluter provided for approximately 5.7 volume replacements per 24-hour period. A photoperiod of 16 hours of light and 8 hours of darkness with a 30-minute transition period was provided. Test temperature was maintained at $20 \pm 2^{\circ}\text{C}$ by a temperature controlled water bath.

The water parameters of the well water used as dilution water were, total hardness: 225 - 275 mg/L as CaCO_3 ; total alkalinity: 325 - 375 mg/L as CaCO_3 ; pH: 7.8 - 8.3; dissolved oxygen: 9.2 - 10.1 mg/L; and conductivity: 700 umhos/cm.

C. Dosage: 21-day flow-through life-cycle test.

- D. **Design:** Forty D. magna ($\leq$ 24-hours old) were randomly assigned to each test concentration (10 per replicate) to initiate the test. A control, solvent control and nominal ^{14}C -Fenoxycarb concentrations of 1.0, 1.7, 3.5, 6.0, and 14 ng a.i./L were tested. The test daphnids were uniformly fed an equal volume (20 - 30 mL) per test chamber of an algal suspension (Selenastrum capricornutum) at least three times daily and supplemented with 0.2 mL per test chamber of Tetramin, cereal leaves, and yeast suspension once daily. Survival of the daphnids was recorded daily. After first instar were observed, the reproduction success was measured by counting and discarding the offspring produced in each concentration every Monday, Wednesday, and Friday for the duration of the study.

Test concentrations of ^{14}C -Fenoxycarb were analytically measured on days 0, 4, 7, 14, and 21 of the study through the use of liquid scintillation counting techniques. Water quality parameter of dissolved oxygen and pH were measured on days 0, 4, 7, 14, and 21 in the control, low, middle, and high test concentrations. Temperature measurements of the water bath were made daily with a mercury thermometer and were recorded continuously with a computerized data logger.

- E. **Statistics:** The selected parameters of survival, adult length (pooled) and total young/adult/reproduction day were analyzed using a one-way analysis of variance. When treatment effects were indicated following a significant F-test of the mean square ratios, a multiple means comparison test (i.e., Dunnett's Test) was used to determine which exposure levels differed from the control value.

Percent survival data were transformed for analysis. All differences were considered significant at the 95% confidence level. Total young/adult/reproduction day for each replicate was calculated by dividing the total number of young produced by the total number of adult reproduction days. The number of reproduction days (normally 13-15) were counted from the day instars were first observed, which for this study was Day 8. Adult reproduction days were calculated for each change in survival in order to be corrected for mortality. Total number of adult reproduction days for each replicate was the sum of each adult reproduction day for each change in survival.

12. **REPORTED RESULTS:** The mean measured concentrations of ^{14}C -Fenoxycarb during the 21-day exposure were 1.6, 2.3, 4.5, 6.8, and 17 ng a.i./L. The mean measured concentrations ranged from 113% to 160% of expected nominal concentrations. Statistical analysis of survival for Daphnia magna after a 21-day exposure to ^{14}C -Fenoxycarb indicated that daphnid survival in the test concentrations were not significantly different ($P \leq 0.05$) from the pooled controls (Table 5, attached).

A one-way analysis and Dunnett's multiple means test indicated that the daphnid lengths in the ^{14}C -Fenoxycarb mean measured concentrations of 2.3, 4.5, 6.8 and 17 ng a.i./L were significantly different from the controls (Table 5, attached).

The mean young/adult/reproduction day after 21 days was significantly affected in the mean measured exposure levels of 2.3, 4.5, 6.8, and 17 ng a.i./L (Table 5, attached). Adults with ephippial eggs were observed in both the 6.8 and 17 ng a.i./L test concentrations during the study. No young were observed until 8 days into the study.

Based on the statistical analyses of survival, adult mean length and young/adult/reproduction day from this 21-day Daphnia magna dynamic life cycle study, the MATC limits were estimated to be the ^{14}C -Fenoxycarb mean measured concentrations of 1.6 and 2.3 ng a.i./L.

Water quality parameters of dissolved oxygen and pH were considered adequate for testing. Temperature, which was recorded continuously, remained consistent at 20°C throughout the study. The dissolved oxygen concentration which ranged from 6.8 to 8.2 mg/L, represented 74 to 89% saturation at 20°C . The pH values of the treatment levels were consistent with the control throughout the study, ranging from 8.2 to 8.4.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The Maximum Acceptable Toxicant Concentration (MATC) of ^{14}C -Fenoxycarb was estimated to be $> 1.6 < 2.3$ ng a.i./L mean measured concentration.

A GLP compliance statement was included in the report and the study was audited by a QA unit. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards: Pesticide Programs (40 CFR 160).

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

- A. Test Procedure: At the present time, there is no SEP for Daphnia magna flow-through chronic test. Therefore, the ASTM Guidelines were used for this data validation. The report deviated from the ASTM as follows:
- o The ASTM states that hardness, alkalinity, and conductivity in the control, low, medium and high concentration test solutions must be measured weekly during the test. This test did not measure these water quality parameters.
 - o The ASTM states that measured concentration of test material in any chamber should be no more than 30% higher or lower than the nominal concentration. If the concentration of the test chamber is too high, the stock solution of test solution may have been prepared incorrectly. All measured concentrations for the lowest concentration (1.0 ng/L) were consistently high, ranging from 130% to 190% of the nominal concentrations. The measured concentrations for 1.7 ng a.i./L ranged from 118% to 165% of the nominal concentration. The measured concentrations for 3.5 ng ranged from 111% to 149% of the nominal concentrations. The highest concentration (14 ng a.i./L) ranged from 107% to 136% of the nominal concentration. Furthermore, the stock solution ranged from 101% to 154% of the nominal concentration (Table 2, attached), which indicates an incorrect stock solution.
- B. Statistical Analysis: The reviewer evaluated daphnid survival following an arc sine square root transformation of the data. The growth (adult length) and reproduction data (# young/adult/reproduction day) were statistically evaluated by ANOVA without any transformations.

The reviewer confirmed that survival for Daphnia magna after 21-days of exposure to ¹⁴C-Fenoxycarb in any test concentration was not significantly different (P = 0.05) when compared to the solvent control.

The reviewer also confirmed a significant difference at P = 0.01 of reproduction (# young/adult/reproduction day) in the four highest concentrations (2.3, 4.5, 6.8, and 17 ng a.i./L) when compared to the solvent control.

The author analyzed the length data using the mean of each replicate instead of individual measurements, thus ignoring the variability among the daphnids within each replicate. However, the analysis performed by the reviewer using individual lengths yielded the same results (i.e., lengths at the four highest treatment concentrations were different from those in the control and solvent control).

The maximum acceptable toxicant concentration (MATC) of ¹⁴C-Fenoxycarb for Daphnia magna was estimated to be > 1.6 ng a.i./L and < 2.3 ng a.i./L mean measured concentration.

C. Discussion/Results: The study results appear scientifically valid. The maximum acceptable toxicant concentration (MATC) of ¹⁴C-Fenoxycarb for Daphnia magna was estimated to be > 1.6 ng a.i./L < 2.3 ng a.i./L mean measured concentration.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 05-03-89.

TABLE 2

Measured Concentrations of ^{14}C -Fenoxycarb During The 21-Day
Chronic Life Cycle Toxicity Study with Daphnia magna

	Measured Concentrations (ppt) ^b						% Nominal
	Day 0	Day 4	Day 7	Day 14	Day 21	Mean ($\pm$ S.D.)	
Level #1 (1.0 ppt) ^a	1.6	1.9	1.3	1.4	1.7	1.6 ($\pm$ 0.24)	160
Level #2 (1.7 ppt) ^a	2.4	2.3	2.0	2.0	2.8	2.3 ($\pm$ 0.33)	135
Level #3 (3.5 ppt) ^a	3.9	4.8	4.2	4.3	5.2	4.5 ($\pm$ 0.52)	129
Level #4 (6.0 ppt) ^a	6.5	7.6	6.3	6.8	6.9	6.8 ($\pm$ 0.50)	113
Level #5 (14 ppt) ^a	15	19	16	17	17	17 ($\pm$ 1.5)	121
Stock (0.157 $\mu\text{g/ml}$) ^a	0.165	0.158	0.208	0.228	0.242	0.200 ($\pm$ 0.0374)	127

^a Nominal concentrations.

^b Stock concentrations in $\mu\text{g/ml}$

TABLE 5

Percent Survival, Adult Length and Young/Adult/Reproduction Day of *Daphnia magna*
Continuously Exposed to ¹⁴C-Fenoxycarb During a 21-Day Life Cycle Study

Chamber I.D. (nominal concentrations)	Mean Measured Concentration (ng/l)	Mean Percent Survival	Adult		Mean Young/Adult/ Reproduction Day
			Mean Length (mm)		
Control	---	98	4.0		6.1
Solvent Control	---	98	4.0		6.2
Controls Pooled ^a	---	98	4.0		6.2
Level #1 (1.0 ng/l)	1.6	100	3.9		6.2
Level #2 (1.7 ng/l)	2.3	100	3.8*		4.5*
Level #3 (3.5 ng/l)	4.5	100	3.7*		2.6*
Level #4 (6.0 ng/l)	6.8	100	3.5*		1.8*
Level #5 (14 ng/l)	17	95**	3.4*		0.90*

*Denotes values significantly different ($\alpha=0.05$) from the pooled controls using one-way analysis of variance (ANOVA) and Dunnett's Multiple means test.

**One adult was killed in transfer on day 15 and was included in compound related mortality total.

^aRepresents pooled control and solvent control values. The controls were combined since statistical analysis showed no significant difference between the measured parameters.

14C - Fenoxycarb Length

Analysis of Variance

File: GROWTH

Date: 05-02-1989

FILTER: None

Analysis of Variance

Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	275	7456.8125			
C (CONC)	6	5442.7012	907.1169	130.882	0.0000
R (REP)	3	33.5099	11.1700	1.612	0.1856
CR	18	261.7671	14.5426	2.098	0.0065
Subj w Groups	248	1718.8340	6.9308		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	79.333	6	70.350
2	79.897	7	67.395
3	78.800		
4	77.050		
5	74.625		

Comparison	Tukey-A*	Bon- ferroni	Dunnett
1 < 2			
1 > 3			
1 > 4	0.0100	0.0033	0.0100 *
1 > 5	0.0100	0.0000	0.0100 *
1 > 6	0.0100	0.0000	0.0100 *
1 > 7	0.0100	0.0000	0.0100 *
2 > 3			N.A.
2 > 4	0.0100	0.0000	N.A. *
2 > 5	0.0100	0.0000	N.A. *
2 > 6	0.0100	0.0000	N.A. *
2 > 7	0.0100	0.0000	N.A. *
3 > 4			N.A.
3 > 5	0.0100	0.0000	N.A.
3 > 6	0.0100	0.0000	N.A.
3 > 7	0.0100	0.0000	N.A.
4 > 5	0.0100	0.0012	N.A.
4 > 6	0.0100	0.0000	N.A.
4 > 7	0.0100	0.0000	N.A.
5 > 6	0.0100	0.0000	N.A.
5 > 7	0.0100	0.0000	N.A.
6 > 7	0.0100	0.0000	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

14C-Fenoxycarb Length

Analysis of Variance.

File: GROWTH

Date: 05-02-1989

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors:	C	R	Concentration ⁿ g/L	N	Mean	S.D.
* *				276	75.3768	5.2073
1 *			Solvent Control	39	79.3333	2.4315
2 *			Control	39	79.8974	2.7222
3 *			1.6	40	78.8000	2.9717
* 4 *			2.3	40	77.0500	2.6308
* 5 *			4.5	40	74.6250	2.6765
* 6 *			6.8	40	70.3500	2.8152
* 7 *			17	38	67.3947	2.8715
* 1				70	75.1143	4.9565
* 2				68	74.9706	4.5679
* 3				70	75.7714	5.9908
* 4				68	75.6471	5.3625
1 1				10	77.9000	1.6633
1 2				9	79.5556	1.8782
1 3				10	80.1000	2.9981
1 4				10	79.8000	2.6162
2 1				10	80.3000	2.4060
2 2				10	78.8000	2.7809
2 3				10	81.1000	2.1833
2 4				9	79.3333	3.2767
3 1				10	77.2000	2.2998
3 2				10	77.1000	2.4698
3 3				10	80.6000	2.7568
3 4				10	80.3000	2.6687
4 1				10	78.1000	2.2336
4 2				10	75.2000	2.2509
4 3				10	77.2000	3.1198
4 4				10	77.7000	2.1628
5 1				10	73.9000	2.6437
5 2				10	75.7000	1.5670
5 3				10	75.3000	3.1640
5 4				10	73.6000	2.8363
6 1				10	71.5000	2.2730
6 2				10	70.3000	2.7101
6 3				10	68.8000	3.4897
6 4				10	70.8000	2.2998
7 1				10	66.9000	3.6040
7 2				9	67.8889	2.6194
7 3				10	67.3000	2.7101
7 4				9	67.5556	2.7889

A total of 1 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

Fmax for testing homogeneity of between subjects variances: 5.29
Number of variances= 28 df. per variance= 9.

14C-Fenoxycarb
 Reproduction
 (# young/adult/reprod. day)

Analysis of Variance

File: survival

Date: 05-02-1989

FILTER: None

Post-hoc tests for factor C (COND)

Level	Mean	Level	Mean
1	6.238	6	1.762
2	6.130	7	0.898
3	6.148		
4	4.472		
5	2.595		

Comparison Dunnett

1 > 2	
1 > 3	
1 > 4	0.0100 *
1 > 5	0.0100 *
1 > 6	0.0100 *
1 > 7	0.0100 *
2 < 3	N.A.
2 > 4	N.A.
2 > 5	N.A.
2 > 6	N.A.
2 > 7	N.A.
3 > 4	N.A.
3 > 5	N.A.
3 > 6	N.A.
3 > 7	N.A.
4 > 5	N.A.
4 > 6	N.A.
4 > 7	N.A.
5 > 6	N.A.
5 > 7	N.A.
6 > 7	N.A.

For Dunnett's test only the P-values .05 and .01 are possible
 and only for comparisons with the control mean (level 1).

14C-Fenoxycarb
Reproduction
(#young/adult/reprod. day)

Analysis of Variance

File: survival

Date: 05-02-1989

FILTER: None

N's, means and standard deviations based on dependent variable: REPROD

* Indicates statistics are collapsed over this factor

Factors: C	<u>Concentration ng/L</u>	N	Mean	S.D.
*		28	4.0346	2.1736
1	Solvent Control	4	6.2375	0.3410
2	Control	4	6.1300	0.6584
3	1.6	4	6.1475	0.5091
* 4	2.3	4	4.4725	0.3555
* 5	4.5	4	2.5950	0.2674
* 6	6.8	4	1.7625	0.4406
* 7	17	4	0.8975	0.1305

Fmax for testing homogeneity of between subjects variances: 25.46

Number of variances= 7 df per variance= 3.

Analysis of Variance

Dependent variable: REPROD

Source	df	SS (H)	MSS	F	P
Between Subjects	27	127.5570			
C (COND)	6	123.9034	20.6506	118.693	0.0000
Subj w Groups	21	3.6536	0.1740		

14C-Fenoxycarb Survival

Analysis of Variance

File: survival

Date: 05-02-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.490	6	1.571
2	1.490	7	1.410
3	1.571		
4	1.571		
5	1.571		

Comparison Dunnett

1 < 2	
1 < 3	
1 < 4	
1 < 5	
1 < 6	
1 > 7	
2 < 3	N.A.
2 < 4	N.A.
2 < 5	N.A.
2 < 6	N.A.
2 > 7	N.A.
3 = 4	N.A.
3 = 5	N.A.
3 = 6	N.A.
3 > 7	N.A.
4 = 5	N.A.
4 = 6	N.A.
4 > 7	N.A.
5 = 6	N.A.
5 > 7	N.A.
6 > 7	N.A.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

14C- Fenoxycarb Survival

Analysis of Variance

File: survival

Date: 05-02-1989

FILTER: None

N's, means and standard deviations based on dependent variable: SURV

* Indicates statistics are collapsed over this factor

Factors: C	Concentration ^{ng/L}	N	Mean	S.D.
*		28	1.5248	0.1147
1	Solvent control	4	1.4903	0.1609
2	Control	4	1.4904	0.1609
3	1.6	4	1.5708	0.0000
4	2.3	4	1.5708	0.0000
5	4.5	4	1.5708	0.0000
6	6.8	4	1.5708	0.0000
7	17	4	1.4099	0.1858

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance

Dependent variable: SURV

Source	df	SS (H)	MSS	F	P
Between Subjects	27	0.3550			
C (CONC)	6	0.0962	0.0160	1.300	0.2972
Subj w Groups	21	0.2589	0.0123		

HC- Fenoxycarb Length

Analysis of Variance

File: GROWTH

Date: 05-02-1989

FILTER: None

Post-hoc tests for factor R (REP)

Level	Mean
1	75.114
2	74.971
3	75.771
4	75.647

Comparison	Tukey-A*	Bon- ferroni	Dunnnett
1 > 2			
1 < 3			
1 < 4			
2 < 3			N.A.
2 < 4			N.A.
3 > 4			N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

For Dunnnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).