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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

Phosmet

SUBJECT: Review of Data for ~~Phorate~~ Registration Standard

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THRU: *for* James W. Akerman, Chief *Raymond W Matheny, Acting*
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TO: George Larocca, PM-15
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Ecological Effects Branch (EEB) has completed the review of a Daphnia magna Life Cycle Chronic Toxicity Test submitted by ICI Americas, Inc. under EPA Accession No.406528-01. The following is a brief summary of the review.

The study appears to be scientifically sound but does not currently satisfy the Guideline requirements. Among the discrepancies are: the percent active ingredient of ¹⁴C-IMIDAN is not clear; raw data are not available for analysis; holding period prior to initiating the study is not reported; water hardness was reported to be 206 to 275 mg/L as CaCO₃ (recommended hardness is 160 to 180 mg/L); and the Standard Evaluation Procedure (SEP) recommends that seven beakers at each concentration containing one daphnid each be used for collection of data on survival, growth, and reproduction plus three beakers at each concentration containing five daphnids each for collection of data on survival. This test procedure used 10 daphnids in each of four replicate test chambers per concentration. Submission of required data and an adequate explanation of the discrepancies may result in the study being upgraded and satisfying the requirement.

The authors concluded that the MATC value for Daphnia magna was 1.1 ug/L.



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11. Materials and Methods (Protocols):

Daphnia magna were obtained from the ABC culture that has been maintained since 1977. The strain of Daphnia has been traced back to 1954 - Pennsylvania State University.

Daphnia were tested at 20 ± 2 °C with a photoperiod of 16-hour daylight with a 30-minute transition period. Light intensity was 50 to 70 foot-candles. Daphnia < 24 hours old were selected for testing.

The following description of the test system was copied directly from the submitted study:

"A half-liter proportional diluter system described by Mount and Brungs, utilizing a Hamilton® Micro Lab 420 syringe dispenser, was used for the intermittent introduction of dilution water and ^{14}C -IMIDAN® into the test chambers. The system contained seven sets of four replicate one-liter test chambers, designated as control, solvent control and level #1 through level #5. Flow-splitting chambers were utilized to thoroughly mix and divide each ^{14}C -IMIDAN® concentration for delivery to the test chambers. To minimize turbulence, the influent water was introduced into the test chambers via 14-gauge hypodermic needles. One-liter glass beakers, labeled A, B, C and D, were used as test chambers. These chambers had notched drains which were covered with 50-mesh stainless steel to prevent escape of the test daphnids. The daphnids were placed in each of the quadruplicate chambers. Aerated ABC well water, with the characteristics summarized in Table 1, was delivered to each test chamber at an average rate of 4.1 ml/min, an amount which was sufficient to replace the 1-liter test volume on an average of 5.9 times in a 24-hour period. The test chambers were immersed in a temperature controlled water bath held at 20°C (+2°C). Temperature was recorded continuously with a Rustrak Ranger® Data Logger.

"The proportional diluter system used for the project was calibrated prior to testing by checking volume deliveries from the diluter mixing cell to obtain a dilution factor used for stock preparations.

"The ^{14}C -IMIDAN® test compound was allowed to flow through the test system prior to study initiation in order to equilibrate the test system. After equilibration was analytically verified, the study was initiated on January 11, 1988, by random assignment of 10 first instar Daphnia magna (ABC Lot #87-I-3) to each of the four replicate test chambers. A total of 40 daphnids were exposed to each test level. The loading rate for each test level was ≈ 1 daphnid per 100 ml of dilution water. The daphnids were randomly

placed in the test chambers with the use of a computer generated randomization table.

"The test daphnids were uniformly fed an equal volume per test chamber of an algal suspension (Selenastrum capricornutum) three times daily providing approximately $2-4 \times 10^8$ cells total to each replicate on each day of the study. ABC mass algae cultures were concentrated by centrifuging. A cell count by hemacytometer was made, and from this count the density of concentrate determined for each mass algae culture harvested. Daphnids were supplemented once daily with 0.2 ml per test chamber of a 9.0 mg/ml suspension of Tetramin®, cereal leaves, vitamins and yeast giving a final suspended solids concentration of 1.8 mg/l.

"Survival, abnormal effects and observance of first brood of the organisms were recorded daily throughout the study. 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. Separating instar from adults was accomplished by gently removing adult daphnids from each chamber by means of a smooth glass pipet and pouring remaining water and young daphnids through a 50 mesh stainless steel screen into a one-liter beaker.

"The young collected on the screen were placed in a beaker to be counted before being discarded. The strained water and adult daphnids were returned to their respective chambers and placed back into the test system. Each chamber was cleaned weekly with a nylon-bristled brush and rinsed with ABC well water. The diluter operation was observed at least twice daily during the study.

"Water quality parameters of dissolved oxygen and pH were measured on Days 0, 4, 7, 14 and 21 in alternating, duplicate replicates of 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 Rustrak Ranger® Data Logger temperature recorder. Dissolved oxygen levels were determined with a YSI Model 54 ARC dissolved oxygen meter and probe, while the pH values were measured with a Beckman 39831 electrode and Corning Model 140 pH/mV meter. Well water samples for total organic carbon and suspended solids analysis have been routinely conducted monthly since January, 1987."

12. Reported Results:

Daphnia magna survival after 21 days was significantly different (0.05) from controls at 3.6 µg/L.

At the 1.6 µg/L rate, *Daphnia* lengths were also significantly different than controls. All daphnids had died before day 21 in the 3.6 µg/L chambers.

The 21-day EC₅₀ (based on immobilization) was 2.0 µg/L.

In the controls, first brood occurred (average) in 8 days. At the 3.6 µg/L dose, time to first brood was significantly affected.

"The mean young/adult/reproduction day after 21 days was significantly affected in the mean measured exposure levels of 1.6 and 3.6 µg/l of ¹⁴C-IMIDAN® (Table 3). All instar produced during the study appeared to be normal.

"Based on the statistical analysis of survival, adult mean length, time until first brood and young/adult/reproduction day from this 21-day *Daphnia magna* dynamic life cycle study, the MATC limits were estimated to be the ¹⁴C-IMIDAN® mean measured concentrations of 0.78 and 1.6 µg/l. The MATC value was calculated to be 1.1 µg/l."

13. Study Authors' Conclusions/Quality Assurance Measures:

"Based on the statistical analysis of survival, adult mean length, time until first brood and young/adult/reproduction day from this 21-day *Daphnia magna* dynamic life cycle study, the MATC limits were estimated to be the ¹⁴C-IMIDAN® mean measured concentrations of 0.78 and 1.6 µg/l. The MATC value was calculated to be 1.1 µg/l."

A Quality Assurance statement was included in the report.

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures - This study does not follow the recommended procedures outlined in the July 1986 Standard Evaluation Procedure (SEP) for *Daphnia magna* chronic toxicity testing. Among the discrepancies are: the percent active ingredient of ¹⁴C-IMIDAN is not provided; no raw data are available for analysis; holding period prior to initiating the study is not reported; water hardness was reported to be 206 to 275 mg/L as CaCO₃ (recommended hardness is 160 to 180 mg/L); it is not clear if adults were placed in water having the same concentration while young were being counted; and the SEP recommends that seven beakers at each concentration containing one daphnid each be used for collection of data on survival, growth, and reproduction and three beakers at each concentration containing five daphnids

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

Chamber I.D. (Nominal Concentrations)	Mean Measured Concentration (µg/L)	Adult ^a Mean Length (mm)	Mean ^a Percent Survival	Mean ^a Young/Adult Reproduction Day	Mean Length to First Brood (Days) ^a
Control	---	3.6	92	4.7	8.0
Solvent Control	---	3.6	92	4.7	8.0
Pooled Controls ^b	---	3.6	92	4.7	8.0
Level 1 (0.19 µg/L)	0.17	3.6	95	4.7	8.0
Level 2 (0.38 µg/L)	0.43	3.6	95	4.6	8.0
Level 3 (0.75 µg/L)	0.78	3.6	95	4.8	8.0
Level 4 (1.5 µg/L)	1.6	3.4*	85	3.4*	8.2
Level 5 (3.0 µg/L)	3.6	*	0*	0.63*	8.5*

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

^aMean values calculated directly from raw data.

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

Measured Concentrations of ¹⁴C-IMIDAN® During the 21-Day
Chronic Life Cycle Toxicity Study with Daphnia magna

Sample	Measured Concentrations (mg/L)						% Nominal ^c
	Day 0	Day 4	Day 7	Day 14	Day 21	(+S.D.)	
Solvent Control	< MQL ^b	< MQL ^b	< MQL ^b	< MQL ^b	< MQL ^b	< MQL	---
Level 1 (0.19 ppb) ^a	0.172	0.178	0.178	0.165	0.167	0.17 (+0.006)	89
Level 2 (0.38 ppb) ^a	0.452	0.454	0.498	0.395	0.374	0.43 (+0.050)	113
Level 3 (0.75 ppb) ^a	0.799	0.791	0.846	0.747	0.701	0.78 (+0.055)	104
Level 4 (1.5 ppb) ^a	1.60	1.63	1.82	1.52	1.48	1.6 (+0.13)	107
Level 5 (3.0 ppb) ^a	3.72	3.59	3.79	3.48	3.35	3.6 (+0.18)	120
Diluter Stock (3.0 µg/mL)	30.2	35.2	37.9	36.3	30.1	34 (+3.6)	113

^aNominal concentrations.

^bMQL calculated for each sample day.

^cPercentage rounded to whole number.

Water Quality Measurements During the Chronic Toxicity Test of ¹⁴C-IMIDAN® to Daphnia magna

Study Day	Replicate	Control			Level #1			Level #3			Level #5		
		Temp. ^a °C	D. O. ^b mg/L	pH ^c	Temp. °C	D. O. mg/L	pH	Temp. °C	D. O. mg/L	pH	Temp. °C	D. O. mg/L	pH
0	A	20	9.0	8.1	20	9.0	8.1	20	9.0	8.1	20	9.1	8.1
	C	20	9.0	8.1	20	9.0	8.1	20	9.1	8.1	20	9.1	8.1
4	B	21	9.0	8.2	21	9.0	8.2	21	9.1	8.2	21	9.1	8.2
	D	21	9.0	8.2	21	9.1	8.2	21	9.1	8.2	21	9.1	8.2
7	A	20	8.7	8.2	20	8.7	8.2	20	8.6	8.2	20	8.6	8.2
	C	20	8.6	8.2	20	8.6	8.2	20	8.6	8.2	20	8.5	8.2
14	B	21	8.7	8.1	21	8.7	8.1	21	8.8	8.1	21	8.8	8.1
	D	21	8.8	8.1	21	8.7	8.1	21	8.8	8.1	21	8.8	8.1
21	A	20	8.6	8.1	20	8.6	8.1	20	8.4	8.1	20	8.4	8.1
	C	20	8.6	8.1	20	8.6	8.1	20	8.4	8.1	20	8.4	8.1

^aTemperature—Mercury Thermometer or continuous measuring devise.

^bDissolved oxygen concentrations—dissolved oxygen system (YSI Model 54 arc).

^cpH—pH Probe (Beckman 39831 electrode) used with a Corning Model 140 pH and mV meter.

Note: Dissolved oxygen saturation at the test temperatures of 20 and 20 °C is 8.7 and 8.5 mg/L. This value has been corrected for altitudinal pressure of ABC Laboratories.

procedure used 10 daphnids in each of four replicate test chambers per concentration.

- b. Statistical Analysis - No raw data were provided for analysis.
- c. Discussion/Results - No conclusions can be reached at this time without benefit of raw data and an explanation of the discrepancies.

d. Adequacy of Study

- 1) Category - ~~Supplemental~~
- 2) Rationale - Refer to Section 14C.
- 3) Repairability - May be upgraded with submission of complete data.

Upgraded to Core
B. Montague
see attached supplemented data and stat. analysis

15. Completion of One-Liner:

16. CBI Appendix: N/A