ACUTE TOXICITY OF PYDRAUL MC TO THE WATER FLEA (*Daphnia magna*).

BN-11-412C

TOXICITY TEST REPORT

SUBMITTED TO

MONSANTO COMPANY

ST. LOUIS, MISSOURI

REPORT #BW-78-3-082

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
March, 1978

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INTRODUCTION -

This investigation was performed at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts to determine the susceptibility of the water flea (Daphnia magna) to Pydraul MC in an acute (48-hour), static toxicity test.

MATERIALS AND METHODS

Pydraul MC (BN-77-412C), a viscous green liquid, tested on a product basis, was received from the Monsanto Company, St. Louis, Missouri. Nominal test concentrations are reported as milligrams of test compound per liter of diluent water (mg/ℓ).

Daphnia magna (≤24 hours old) used in this toxicity test were from laboratory stocks cultured at E G & G, Bionomics. Deionized, reconstituted well water with a total hardness of 180 mg/ℓ as $CaCO_3$, a pH of 8.1, a temperature of 22 ± 1°C and a dissolved oxygen (DO) concentration of greater than 60% of saturation was used to culture these animals.

Procedures used in this acute toxicity test were based on protocols in "Methods for acute toxicity tests with fish,

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macroinvertebrates, and amphibians" (U.S. EPA, 1975) except where stated otherwise.

Two independent tests involving two different series of concentrations were performed in this study. A preliminary (range-finding) test was performed to define a narrower range of concentrations to be used in a subsequent definitive test. Mortality data derived from the definitive test were used to calculate a median lethal concentration (LC50) and its 95% confidence limits utilizing linear regression analysis or the moving average angle method (Harris, 1959) where applicable. The LC50 is the calculated nominal concentration of the test compound in diluent water which produces 50% mortality in the test animal population at the stated times of exposure.

The static toxicity tests were conducted in 250 milliliter (mℓ) beakers which contained 150 mℓ of test solution. The diluent water used in this study had the same water quality characteristics as described previously for culture water. For each test concentration, the appropriate amount of the test compound, dissolved in acetone, was pipetted into 500 mℓ of diluent water and mixed with a magnetic stirrer. This solution was then divided into three 150 mℓ aliquots in triplicate beakers to provide replicate exposure treatments. The remaining 50 mℓ were used for 0-hour and DO and pH determina-

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tions. A negative control, consisting of the same dilution water and conditions but with no test compound or acetone, was established. A positive control, consisting of the same dilution water, conditions, and containing the greatest amount of acetone present in any test vessel was also established. All test vessels were maintained at 22 $\pm$ 1°C and test solutions were not aerated during the test. Five daphnids were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 15 daphnids per concentration.

During this test, the dissolved oxygen concentration, pH and temperature of test solutions were monitored at the initiation and termination of the toxicity test in the high, middle and low test concentrations. DO and temperature were measured with a YSI dissolved oxygen meter and combination oxygen-temperature probe. pH was measured with an Orion Specific Ion meter. The total hardness determinations of diluent water were conducted according to "Standard methods for the examination of water and wastewater" (1975).

RESULTS

During the 48-hour toxicity test with Pydraul MC, pH and dissolved oxygen ranged from 8.1 to 8.3 and 7.8 mg/ℓ (89%

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of saturation) to 8.3 mg/ℓ (94% of saturation), respectively. The highest nominal concentration of Pydraul MC at which there was no discernible effect on the test animals during the 48-hour toxicity test was 0.17 mg/ℓ. Table 1 presents a summary of the average observed percentage mortality of each individual test concentration after 24- and 48-hours of exposure. The calculated 24-hour LC50 for Pydraul MC was 3.46 (0.78-15) mg/ℓ according to linear regression analysis. The calculated LC50 value, 95% confidence limits after 48-hours exposure was 0.38 (0.30-0.48) mg/ℓ. The corresponding moving average angle after 48-hours of exposure is presented in Figure 1.

At the test concentration of 4.1 mg/ℓ, the Pydraul MC precipitated on the surface of the test solutions. Test organisms became entrapped at the air-water interface at all Pydraul MC test concentrations.

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LITERATURE CITED

APHA, AWWA, WPCF. 1971. EDTA Titrimetric Method. Standard Methods. pp. 179-184.

Harris, E.K. 1959. Confidence limits for the LD50 using the moving average-angle method. Biometrics, Vol. 4, #3. pp. 157-164.

U.S. EPA. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 pp.

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Table 1 -- Concentrations tested and corresponding observed percentage mortalities for the water flea (Daphnia magna) exposed to Pydraul MC.

Nominal concentration (mg/ℓ)	Average percentage mortality							
	24-hour				48-hour			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
4.1	60	60	40	53	100	100	100	100
2.2	40	0	40	27	100	100	100	100
1.1	20	20	0	13	100	100	80	93
0.60	0	0	20	7	100	100	60	87
0.32	0	0	0	0	60	20	40	40
0.17	0	0	0	0	0	0	0	0
control (negative)	0	0	0	0	0	0	0	0
control (positive)	0	0	0	0	0	0	0	0

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Sample:	Batch #	% active	Date received	Water
Pydrawl MC	—	tested as 100%	1/31/3	recon ≈ 170 mg/l $CaCO_3$

Temp.	Test Date	Starting hour	Technician	species	Size/age
22°C	1/26/78	11 AM	Brenda	D. magna	≤ 24 hours old

Used	Condition	Source	Solvent	test vessel	Formulation
5/rep 3 reps	good	cultures	acetone	250 ml beakers	see reverse side

INVERTEBRATE TOXICITY TEST - E G & G BIONOMICS

Concentration	% MORTALITY-IMMOBILIZATION											
	0 hr.		24 hr.				48 hr.				48 hr.	
(mg/l)	D.O.	P.H.	a	b	c	$\bar{x}$	a	b	c	$\bar{x}$	D.O.	P.H.
CONTROL	7.8	8.1	0	0	0	0	0	0	0	0	8.3	8.3
solvent control	8.0	8.2	0	0	0	0	0	0	0	0		
4.1	7.9	8.2	3	3	2	53	5	5	5	100	8.3	8.2
2.2			2	0	2	27	5	5	5	100		
1.1	8.1	8.2	1	1	0	13	5	5	4	93	8.2	8.2
0.60			0	0	1	7	5	5	3	87		
0.32			0	0	0	0	3	1	2	40		
0.17	8.0	8.2	0	0	0	0	0	0	0	0	8.3	8.3
✓						✓				✓		

OBSERVATIONS

Conc: (g/l)	Cloudy	percipitate	# lethargic animals	# animals on surface	Comments
CONTROL					
solvent Control					
4.1		24 hr - on surface 48 " " "	24 - C-3 B-2 A-3	24 - C-5 B-5 A-5	
2.2			24 - A-3 B-5 C-3	24 - A-5 B-5 C-5	
1.1			24 - C-5 B-4 A-4	24 - A-4 B-5 C-5	
0.60			24 - C-1 B-2 A-3	24 - C-3 B-3 A-4	
0.32			24 - B-2 Caught on bottom	24 - C-2 B-1 A-1	
0.17			48 - B-1	48 - B-1	

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stock: 0.8.100 g 100 ml acetone → 8.. mg/ml

conc (ng/l)	ml stock	ml H_2O
4.1	0.25	500
2.2	0.13	500
1.1	0.067	500
0.60	0.036	500
0.32	0.02	500
0.17	0.01	500

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Sample:	Batch #	% active	Date received	Water
NC	—	100	1/3/78	Recon ≈ 170mg/l $CaCO_3$

Temp.	Test Date	Starting hour	Technician	species	Size/age
22°C	1/24/78	3:00	BW	D. magna	≤ 24 hours old

Used	Condition	Source	Solvent	test vessel	Formulation
5/rep 3 reps	good	cultures	acetone	250ml beakers	see reverse side

INVERTEBRATE TOXICITY TEST - E G & G BIONOMICS

Concentration (mg/l)	0 hr. D.O.	0 hr. P.H.	% MORTALITY-IMMOBILIZATION 24 hr. a	24 hr. b	24 hr. c	24 hr. $\bar{x}$	48 hr. a	48 hr. b	48 hr. c	48 hr. $\bar{x}$	48 hr. D.O.	48 hr. P.H.
CONTROL			0	0	0	0	0	0	0	0		
10			2	3	0	33	5	5	5	100		
1.0			0	0	0	0	4	4	4	60		
SOLVENT CONT			0	0	0	0	0	0	0	0		

OBSERVATIONS

Conc: (mg/l)	Cloudy	percipitate	# lethargic animals	# animals on surface	Comments
CONTROL					
10		24-c/cloudy white oil/slick on surface 48- " " "	24- A-3 B-2 C-5	24-A-5 48A-5 24-B-5 B-5 C-5 C-5	
1		24-oil/slick on surface 48- " "	24-A-4 48-C-4 C-2 B-1 a	24-A-4 48A-5 B-5 B-5 C-5 C-5	24-A-could only find 4 animals 48-C-4th animals laying on b
control + acetone					

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Stock 2g / 100-ml acet → 20mg/ml

conc (mg/l)	ml stock	ml HOH
10	0.25	500 ml
1.0	0.025	500 ml

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