RAW DATA ANALYSIS

TEST MATERIAL: Pydraul MC

REPORT NUMBER: BN-77-412A

TYPE OF STUDY: LC_{50}

TEST ORGANISM: Rainbow Trout

DATE AUDITED: 4/12/78

PARAMETERS REVIEWED:

- ✓ Test Procedure
- ✓ Test Concentrations (Volume of Test Material added / Dilution Water Volume)
- ✓ Data Consistent with Final Report
- ✓ Calculations Accurate
- ✓ Other: pH, DO, mortality, behavior

DISCREPANCIES:

DO too low at 96 hours?

AUDITED BY: Rita M. Comotto

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ACUTE TOXICITY OF PYDRAUL® MC (BN-77-412A) TO RAINBOW TROUT (*Salmo gairdneri*).

TOXICITY TEST REPORT

SUBMITTED TO

MONSANTO COMPANY

ST. LOUIS, MISSOURI

REPORT #BW-78-3-092

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

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INTRODUCTION

A 96-hour, static, acute toxicity determination was performed at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts to determine the acute toxicity of Pydraul® MC (BN-77-412A) to rainbow trout (Salmo gairdneri).

MATERIALS AND METHODS

Procedures used in the acute toxicity determination followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975).

Pydraul® MC (BN-77-412A), a green viscous liquid, was tested as received from the Monsanto Company, St. Louis, Missouri. Nominal test concentrations are reported as milligrams of Pydraul® MC per liter of test solution (mg/ℓ).

Rainbow trout used were obtained from a commercial fish hatchery in Washington. Upon receipt at Bionomics, the fish were held in a 1700-ℓ circular metal pool that had previously been coated with an epoxy resin paint to prevent leaching of extraneous materials into the water. Well water characterized as having a total hardness and alkalinity as calcium carbonate ($CaCO_3$) of 32 mg/ℓ and 20 mg/ℓ, respectively (APHA, et al.,

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1975), a pH of 7.1 and a temperature of 11 ± 1°C flowed through the pool at a minimum of 4 l/minute. This flow rate was adequate to maintain a dissolved oxygen (DO) concentration greater than 70% of saturation. Experimental animals were maintained under these conditions for a minimum of 14 days. During this time period, the observed cumulative mortality of the fish was less than 2% and they were judged to be in excellent condition. All fish were fed a dry, pelleted food, ad libitum, daily and ground liver weekly except during the 48-hours prior to testing.

Two independent tests involving two different series of concentrations were performed for this toxicity determination. The mortality data derived from a preliminary range-finding test were used to define a narrower series of concentrations to be used in the subsequent definitive test.

The tests were conducted in 19.6-ℓ glass jars which contained 15 ℓ of test solution. The diluent water used was soft water reconstituted according to recommended procedures (U.S. EPA, 1975). This water was characterized as having a total hardness and alkalinity as $CaCO_3$ of 40 mg/ℓ and 31 mg/ℓ, respectively (APHA et al., 1975), a pH of 7.6 and a conductivity of 159 micromhos per centimeter. A concentrated stock solution of Pydraul® MC was prepared by mixing 3.4 grams of the test material

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in 100 milliliters (mℓ) of acetone, resulting in a 34 mg/mℓ suspension. This suspension was shaken to insure homogeneity and the appropriate amount was pipetted directly into each test jar and mixed by stirring.

Control and solvent control jars were also established. Both jars contained the same dilution water as used in the exposure jars but contained no Pydraul® MC, while the solvent control also contained 2.3 mℓ of acetone. All test solutions were maintained at 12 ± 1°C and were not aerated.

Ten rainbow trout with a mean and standard deviation (N=30) wet weight of 2.0 ± 1.0 grams and a mean standard length of 48 ± 8 millimeters were randomly distributed to each test jar within 30 minutes after the test compound was added.

During the study, the pH and dissolved oxygen concentrations were measured at 0-, 24-, 48- and 96-hours in the control, high, middle and low test concentrations. The pH was measured with an Instrumentation Laboratory pH meter and combination electrode, and the temperature and DO were measured with a YSI combination oxygen-temperature meter and probe.

A 50 mℓ sample was taken from the 1.9 mg/mℓ concentration of Pydraul® MC at 96 hours and preserved with 10 mℓ of hexane and

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stored for future shipment to Monsanto Company at the request of Ms. Rita Comotto.

Test concentrations and corresponding percentage mortality data derived from the definitive test were used to calculate 24-, 48-, 72- and 96-hour median lethal concentrations (LC50's) and 95% confidence intervals by means of the moving average angle method (Harris, 1959). The LC50 is defined as the calculated nominal concentration of the test compound in diluent water which caused 50% mortality in the test animal population at the stated exposure interval.

Prior to analysis by this method, nominal compound concentrations were transformed to logarithms and corresponding percentage mortalities to angles. Each group of three successive angles was then averaged and the LC50 was estimated by linear interpolation between the successive concentrations whose average angles bracketed 45°.

RESULTS

The 96-hour LC50 for rainbow trout exposed to Pydraul® MC was 1.8 mg/ℓ. The pH and DO concentrations measured during the 96-hours of exposure are presented in Table 1. The calculated LC50 values and 95% confidence intervals for 24-, 48-,

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72- and 96-hours are presented in Table 2. Table 3 presents a summary of percentage mortalities observed in each individual test concentration at the 24-, 48-, 72- and 96-hour exposure intervals. Also presented in Table 3 are the observed behavioral responses of the fish. All test concentrations turned cloudy immediately after treatment with the stock solution.

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LITERATURE CITED

APHA, AWWA, WPCF. 1975. Standard Methods for the examination of water and wastewater. 14th Edition, Washington, D.C. 1193 pp.

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 -- pH and DO concentrations measured during the 96-hour toxicity test with rainbow trout (Salmo gairdneri) exposed to Pydraul® MC.

	Nominal concentration (mg/ℓ)	0-hour	24-hour	48-hour	96-hour
pH	5.3	7.4	7.0	6.8	6.8
	1.9	7.5	7.0	6.6	6.7
	0.41	7.5	7.0	6.6	6.7
	control (acetone)	7.4	7.0	6.7	6.6
	control	7.5	7.1	6.9	6.8
DO (mg/ℓ)	5.3	8.0	5.3	4.7	3.4
	1.9	8.2	4.4	2.8	2.4
	0.41	8.0	4.9	2.5	2.2
	control (acetone)	7.8	4.2	2.4	1.8
	control	7.9	6.0	3.8	2.6

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Table 2 -- Acute toxicity of Pydraul® MC to rainbow trout (Salmo gairdneri) during the 96 hours of exposure.

LC50 (milligrams/liter)				No discernible effect level at 96 hours (mg/ℓ)
24-hour	48-hour	72-hour	96-hour	
2.4 (1.8-3.0)[a]	1.8 (1.5-2.0)	1.8 (1.5-2.0)	1.3 (1.6-2.0)	<0.41

[a] 95% confidence interval.

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Table 3 -- Concentrations tested and corresponding percentage mortalities of rainbow trout (Salmo gairdneri) observed after 24-, 48-, 72- and 96-hours exposure to Pydraul® MC.

Nominal concentration (mg/ℓ)	% mortality			
	24-hour	48-hour	72-hour	96-hour
5.3	100	100	100	100
3.2	90[a]	100	100	100
1.9	10[a,b]	10[a,b]	10[a,c,d]	20[c,d]
1.1	10	10[b]	10[c,d]	10[c,d]
0.68	0[a]	0[a]	0[c,d]	0[a,c,d]
0.41	0[b]	0	0	10
control (acetone)	0	0	0	0
control	0	0	0	0

[a] Some fish showed a complete loss of equilibrium.
[b] Some fish showed a dark coloration.
[c] All fish showed a dark coloration.
[d] All fish were at the surface.

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COMPOUND/% A.I.	COMPANY	LOT #	DATE TEST STARTED: 2/13/78	DATA BY
Pydrall MC /100%	Monsanto	BN 77-412	TIME TEST STARTED: 4:15	Charlotte Diefel

SPECIES	SOURCE	MEAN (N=30)	STOCK CONC.	SOLVENT	CONTROL JAR TEMP. °C
Salmo gairdneri	Tank P2 Lot # 77A36 State Wash	length 48 ± 8.4 mm weight 1.95 ± 1.03 g	34 mg/ℓ (mg/mℓ)	Acetone	0 13° 24 12.5° 48 12° 72 11° 96 12° / hour

DILUTION WATER

SOURCE Recon TYPE RC 56 pH 7.6 HARDNESS (mg/ℓ $CaCO_3$) 40 ALKALINITY (mg/ℓ $CaCO_3$) 31 CONDUCTIVITY (µmhos/cm) 159 SALINITY (o/oo) ____

Test Container Volume = 19.6 ℓ
Diluent Volume = 15.0 ℓ

10 animals / 1 replicate

sampled 1.9 mg/ℓ

COMMENTS: 96 hr H_2O samples.

H_2O cloudy after addition of stock.
ok SJC 2/20/78

Rbk

PERCENTAGE MORTALITY

Apparent 96-Hour No Effect Level <.41 mg/ℓ

CONC. µg/ℓ g/ℓ mg/ℓ %	Amount required (mℓ)	0-hour CS DO	0-hour pH	24-hour CS # dead	24-hour Obser.	24-hour DO	24-hour CS pH	48-hour CS # dead	48-hour Obser.	48-hour DO	48-hour DMS pH	72-hour DO # dead	72-hour Obser.	72-hour DO	72-hour pH	96-hour CS # dead	96-hour Obser.	96-hour DO	96-hour DM pH
Control		7.9	7.5	0		6.0	7.1	0		3.8	6.9	0				0		2.6	6.
Sol. control acetone	2.3	7.8	7.4	0		4.2	7.0	0		2.4	6.7	0				0		1.8	6.
5.3	2.3	8.0	7.4	10		5.3	7.0	0		4.7	6.8	0				0		3	6.8
3.2	1.4			9	1 CLE			1				0				0			
1.9	.84	8.2	7.5	1	1 CLE 2 Drk	4.4	7.0	0	1 CLE 2 Drk	2.8	6.6	0	2 cle as drk			1	as drk	2.4	6.7
1.1	.49			1				0	1 Drk			0	drk as			0	AS Drk		
0.68	.30			0	1 CLE			0	1 CLE			0	drk as			0	AS Drk 1 CLE		
0.41	.18	8.0	7.5	0	1 Drk	4.9	7.0	0		2.5	6.6	0				1		2.2	6.7

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OBSERVATIONS

General Behavior

CLE - Complete loss of equilibrium

PLE - Partial loss of equilibrium

HYP - Hyperactive

LETH - Lethargic

PFAE - Pectoral fins anteriorly extended

EXO - Exopthalamus

EA - Extended abdomen

SU - Surfacing

SO - Sounding

TW - Twitching

AS - At the surface

Swimming

ERR - Erratic

GY - Gyrating

SK - Skattering

IN - Inverted

Pigmentation

DRK - Dark

Integument

MS - Mucous shedding

EMP - Excessive mucous production

Respiration

RA - Rapid

SL - Slow

GA - Gulping air

Solution

CLDY - Cloudy

PRE - Precipitate

FOS - Film on surface

UC - Undissolved chemical

Diane M. Sullivan - DMS

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